Supplementary

Table S1 Summary of MeDIP-seq results

Twin pair ID	Gender	Neonate ID	Gestational age (weeks)	Birth weight (g)	Number of total reads	Number of aligned reads	Alignment rate	Number of mapped reads[†]	Number of filtered reads[‡]	Final mapping rate	Length of mapped fragments[§]	PDC normalization factor[¶]
P1	F	P1L	37	1,850	47,838,096	422,979,43	88.42%	28,285,910	28,283,845	59.12%	250±41	0.3389
		P1H		2,650	48,979,592	43,617,824	89.05%	29,934,212	29,932,689	61.11%	272±41	0.3361
P2	F	P2L	35	2,400	48,979,592	36,167,010	73.84%	22,074,160	22,072,287	45.06%	260±44	0.4813
		P2H		2,450	48,979,592	37,701,789	76.97%	21,783,485	21,781,786	44.47%	268±42	0.5476
P3	M	P3L	37	2,150	44,408,428	33,725,613	75.94%	21,109,901	21,108,317	47.53%	257±41	0.5113
		P3H		2,850	48,979,592	37,593,844	76.75%	22,680,424	22,679,036	46.30%	279±42	0.4726
P4	M	P4L	36	2,200	48,979,592	39,109,440	79.84%	23,955,535	23,953,655	48.91%	255±45	0.4816
		P4H		2,300	48,979,592	37,415,496	76.39%	21,899,257	21,897,831	44.71%	271±45	0.5200

[†], a mapped read is a paired-end read whose two mates aligned concordantly and uniquely to the reference genome. [‡], two or more paired-end reads that aligned to the exact same genomic position were considered PCR duplicates and were removed. [§], values are provided as "mean ± standard deviation". [¶], the normalization factor for precipitated DNA coverage, which is the total length of all promoter regions divided by the number of residues in the uniquely mapped fragments that fell into any promoter region.

Table S2 Top 50 differentially methylated genes (DMGs)

Rank	Gene symbol	Chr	DMPR location[†]	Change direction[‡]	Q1 significance[§]	Q2 significance[¶]	Gene function
1	*TRAF3IP2*	chr6	111927086	+	3.20E−11	0.0172	Inflammation and cytokine signaling
2	*TTN*	chr2	179670753	+	3.68E−11	2.23E−05	Neurodevelopment and cognition
3	*LRRC3B*	chr3	26701271	+	4.72E−10	0.0034	Function unclear
4	*ZCCHC16*	chrX	111440315	–	5.31E−09	0.0202	Function unclear
5	*BACH2*	chr6	90879731	–	1.54E−08	0.0189	Cell cycle and genomic stability
6	*FHIT*	chr3	60726568	+	1.83E−08	0.0299	Cell cycle and genomic stability
7	*CENPL*	chr1	173786932	+	2.88E−08	0.0283	Cell cycle and genomic stability
8	*RFX3*	chr9	3459238	–	5.72E−08	0.0085	Cell cycle and genomic stability
9	*INPP4B*	chr4	143703559	–	8.49E−08	1.76E−04	Metabolism and energy homeostasis
10	*PDE4D*	chr5	59637235	+	8.80E−08	0.0132	Metabolism and energy homeostasis
11	*C3orf67*	chr3	58970029	–	9.32E−08	0.0025	Cell cycle and genomic stability
12	*ZNF438*	chr10	31186476	+	9.89E−08	5.29E−04	Neurodevelopment and cognition
13	*FBXL19*	chr16	30935006	+	1.04E−07	0.0237	Cell cycle and genomic stability
14	*AFTPH*	chr2	64769430	–	1.28E−07	5.42E−04	Cell cycle and genomic stability
15	*ADAMTSL3*	chr15	84321788	+	1.37E−07	0.0238	Metabolism and energy homeostasis
16	*C1orf129*	chr1	170906500	+	1.38E−07	0.0172	Cell cycle and genomic stability
17	*LEPR*	chr1	65997970	+	1.66E−07	0.0227	Metabolism and energy homeostasis
18	*ZFP2*	chr5	178349249	–	1.83E−07	0.0111	Neurodevelopment and cognition
19	*TMEM178*	chr2	39924620	+	1.95E−07	0.0089	Function unclear
20	*RABGAP1L*	chr1	174172053	+	2.12E−07	0.0296	Function unclear
21	*PPP2R2B*	chr5	146328857	–	2.54E−07	0.0011	Metabolism and energy homeostasis
22	*OR5L1*	chr11	55578613	+	2.59E−07	0.0021	Function unclear
23	*GLIS3*	chr9	4288234	+	2.62E−07	0.0219	Metabolism and energy homeostasis
24	*C1orf94*	chr1	34655361	+	2.73E−07	0.0262	Cell cycle and genomic stability
25	*MYT1L*	chr2	2217117	+	4.34E−07	0.0082	Neurodevelopment and cognition
26	*GPBP1L1*	chr1	46132588	+	5.71E−07	0.0168	Function unclear
27	*RNF19A*	chr8	101316365	–	6.04E−07	0.0038	Inflammation and cytokine signaling
28	*TFAP2D*	chr6	50680589	–	9.17E−07	0.0097	Function unclear
29	*PLEKHG1*	chr6	150980644	+	9.71E−07	0.0197	Function unclear
30	*C4orf19*	chr4	37492725	+	1.14E−06	0.0215	Cell cycle and genomic stability
31	*LRRC4C*	chr11	40292322	+	1.22E−06	0.013	Neurodevelopment and cognition
32	*NRXN3*	chr14	78945709	–	1.23E−06	0.0016	Neurodevelopment and cognition
33	*PACSIN2*	chr22	43384524	–	1.28E−06	0.023	Inflammation and cytokine signaling
34	*OSR2*	chr8	99958961	–	1.31E−06	0.0276	Function unclear
35	*PVRIG*	chr7	99816595	+	1.32E−06	0.0144	Function unclear
36	*FAM135A*	chr6	71125001	–	1.39E−06	0.0037	Function unclear
37	*PLCB4*	chr20	9055098	+	1.42E−06	0.0088	Metabolism and energy homeostasis
38	*NLGN4X*	chrX	6071112	–	1.45E−06	0.0025	Neurodevelopment and cognition
39	*MC3R*	chr20	54823629	–	1.57E−06	8.13E−04	Metabolism and energy homeostasis
40	*FBXW7*	chr4	153393902	+	1.86E−06	4.29E−04	Inflammation and cytokine signaling
41	*JAK1*	chr1	65383795	+	1.91E−06	0.0013	Inflammation and cytokine signaling
42	*PACSIN1*	chr6	34434608	+	2.04E−06	0.0234	Inflammation and cytokine signaling
43	*FAM189B*	chr1	155225481	+	2.23E−06	0.0106	Function unclear
44	*TXNDC15*	chr5	134209338	–	2.53E−06	0.0243	Inflammation and cytokine signaling
45	*PRICKLE4*	chr6	41747752	+	2.55E−06	0.0293	Neurodevelopment and cognition
46	*FRS3*	chr6	41747752	+	2.55E−06	0.0293	Neurodevelopment and cognition
47	*C5orf34*	chr5	43515821	+	2.80E−06	0.0268	Cell cycle and genomic stability
48	*ZBTB20*	chr3	114077642	+	2.82E−06	0.0075	Neurodevelopment and cognition
49	*MAP2*	chr2	210414237	+	2.96E−06	0.0294	Neurodevelopment and cognition
50	*GTDC1*	chr2	144997837	–	3.40E−06	0.0291	Cell cycle and genomic stability

The highlighted 11 DMGs participate in biological processes through functional interactions. [†], the center base location of the most significant differentially methylated 500 bp region in this gene promoter. [‡], change directions are annotated referencing the higher birth-weight group. [§], testing of the null hypothesis that this region is not differentially methylated between the lower birth weight group and the higher birth weight group. [¶], testing of the null hypothesis that within twin pairs, methylation change in this region does not indicate birth weight difference. Cutoff: <0.03.

Table S3 The relative mRNA levels of 11 DMGs in cord blood mononuclear cells (CBMCs) from independent neonates

Gene	Low birth weight (LBW) group	Normal birth weight (NBW) group	P value (ANCOVA)
NRXN3	2.593±1.640	1.170±0.765	0.005
RNF19A	1.356±0.402	0.926±0.244	<0.001
RFX3	1.349±0.584	1.010±0.397	0.021
JAK1	0.610±0.230	1.124±0.416	<0.001
FRS3	0.828±0.455	1.107±0.263	0.021
FBXW7	0.800±0.322	1.137±0.479	0.008
PPP2R2B	1.781±2.005	1.914±1.778	0.363
FHIT	1.026±0.381	0.899±0.242	0.326
LEPR	1.376±0.632	1.069±0.532	0.077
NLGN4X	0.676±0.627	1.173±0.915	0.021
PACSIN2	0.561±0.279	1.060±0.597	0.002

Values are provided as mean ± standard deviation. Analysis of covariance (ANCOVA) was used to adjust for gender, gestational age, and mode of delivery in the comparison between the LBW and NBW groups.

Table S4 The relative mRNA levels of cytokine responses to immune stimulants in neonatal cord blood monocytes and lymphocytes

Cytokine	Stimulant	Measurement time (hour)	Stimulant concentration (μg/mL)	Low birth weight (LBW) group	Normal birth weight (NBW) group	P value (ANCOVA)
IFN-γ	PHA	0	0	0.0012±0.0029	0.0004±0.0007	0.075
		24	1	0.0100±0.0183	0.0020±0.0036	<0.001
IP-10	PHA	0	0	0.0020±0.0074	0.0005±0.0006	0.207
		24	1	0.0230±0.0394	0.0020±0.0032	<0.001
IL-4	PHA	0	0	0.0001±0.0002	0.0019±0.0078	0.259
		24	1	0.0012±0.0032	0.0070±0.0150	0.002
IL-5	PHA	0	0	0.0006±0.0009	0.0006±0.0012	0.783
		24	1	0.0027±0.0043	0.0017±0.0033	0.607
IL-10	PHA	0	0	0.0023±0.0029	0.0154±0.0675	0.155
		24	1	0.0045±0.0037	0.0315±0.0544	0.003
IL-1β	LPS	0	0	6.9053±4.4400	3.8255±3.1665	<0.001
		3	0.1	20.5092±17.5239	9.9478±5.1696	<0.001
IL-6	LPS	0	0	0.2215±0.1822	0.2311±0.2293	0.464
		3	0.1	0.6642±0.5833	0.5994±0.4073	0.861
TNF-α	LPS	0	0	0.1686±0.1665	0.1064±0.1360	0.046
		3	0.1	0.3881±0.3329	0.2297±0.2173	0.006

Values are provided as mean ± standard deviation. Analysis of covariance (ANCOVA) was used to adjust for gender, gestational age, and mode of delivery in the comparison between the LBW and NBW groups.

Table S5 Supernatant protein levels of cytokine responses to immune stimulants in neonatal cord blood monocytes and lymphocytes

Cytokine	Stimulant	Measurement time (hour)	Stimulant concentration (μg/mL)	Low birth weight (LBW) group (pg/mL)	Normal birth weight (NBW) group (pg/mL)	P value (ANCOVA)
IFN-γ	PHA	0	0	853.774±173.926	796.922±174.026	0.114
		48	1	1052.778±138.694	988.544±117.836	0.002
IP-10	PHA	0	0	89.191±16.261	85.943±17.296	0.414
		48	1	104.653±13.740	109.106±16.294	0.182
IL-4	PHA	0	0	281.318±59.668	278.073±58.420	0.968
		48	1	340.428±65.038	342.214±59.950	0.403
IL-5	PHA	0	0	170.388±35.624	164.220±31.831	0.716
		48	1	208.447±28.536	190.897±31.199	0.008
IL-10	PHA	0	0	234.716±37.741	227.707±42.263	0.222
		48	1	276.193±40.730	270.366±41.303	0.939
IL-1β	LPS	0	0	16.573±3.364	16.579±3.739	0.831
		12	0.1	20.741±2.838	20.359±3.255	0.637
IL-6	LPS	0	0	57.296±10.633	55.536±11.208	0.424
		12	0.1	70.557±9.806	66.076±10.452	0.032
TNF-α	LPS	0	0	281.328±57.540	265.281±59.148	0.379
		12	0.1	337.190±55.145	343.240±59.724	0.523

Values are provided as mean ± standard deviation. Analysis of covariance (ANCOVA) was used to adjust for gender, gestational age, and mode of delivery in the comparison between the LBW and NBW groups.

https://dx.doi.org/10.21037/tp-2025-1-954