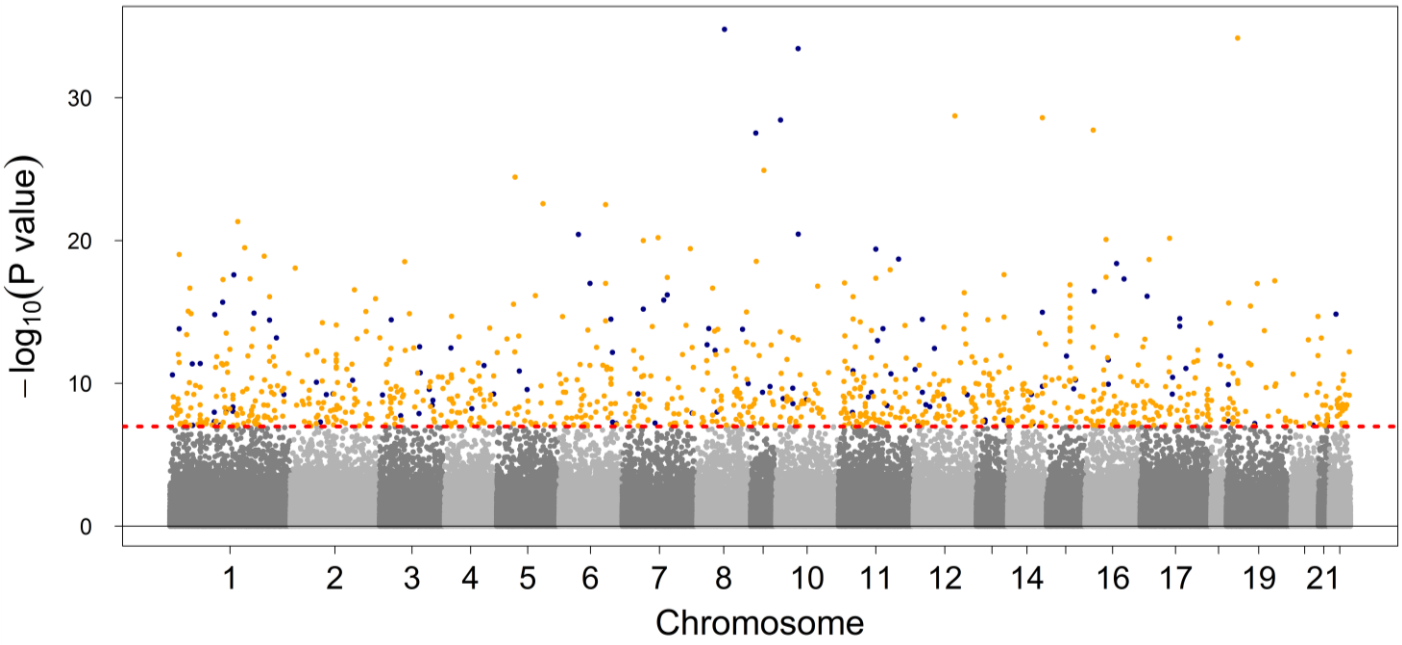
Supplementary Information

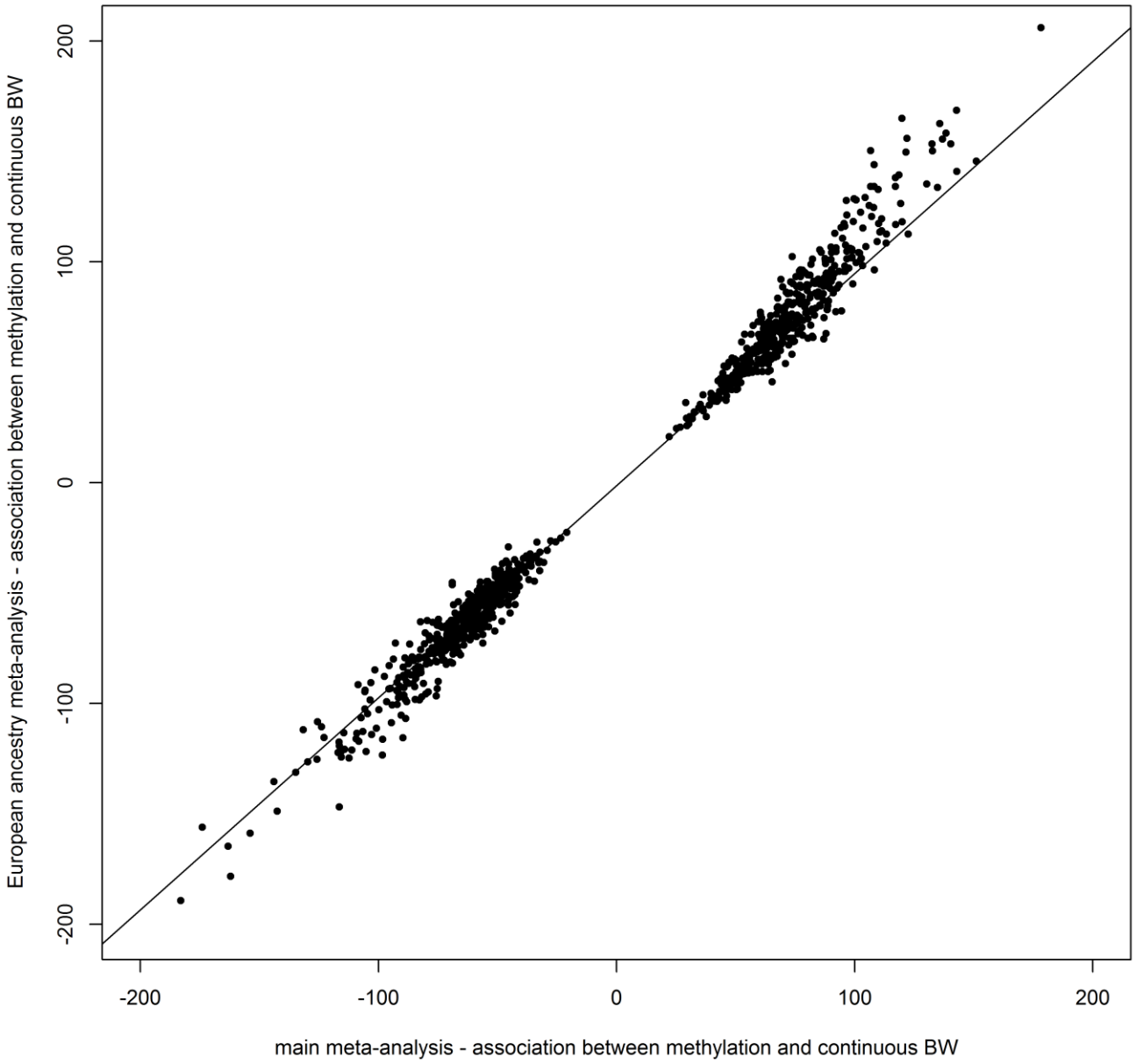
Meta-analysis of epigenome-wide association studies in neonates reveals widespread differential DNA methylation associated with birthweight.

Küpers L.K. *et al*.



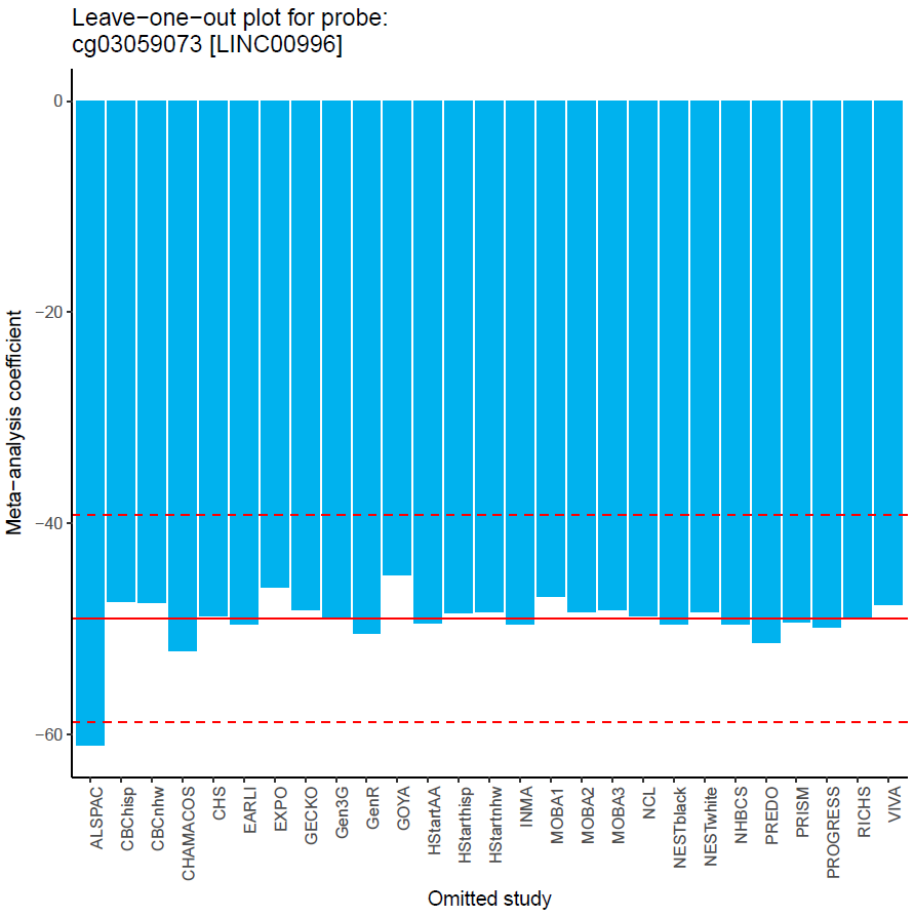
Supplementary Figure 1. Manhattan plot showing the main meta-analysis results for associations between DNA methylation and birthweight as a continuous measure

The red dashed line shows the Bonferroni-corrected significance threshold for multiple testing ($p<1.06x10^{-7}$). Highlighted in orange are the 914 CpGs with $p<1.06x10^{-7}$ and $I^2 \leq 50\%$ and highlighted in blue are the 115 CpGs with $p<1.06x10^{-7}$ and $I^2>50\%$.



Supplementary Figure 2. Correlation of the point estimates for the secondary meta-analysis of European ancestry only (y-axis) versus main meta-analysis (x-axis)

Correlation of the point estimates for a secondary meta-analysis of 6,023 participants from 17 studies who were all of European ancestry (y-axis) with point estimates for the main meta-analysis with 8,809 participants from 24 studies of mixed ancestry (x-axis).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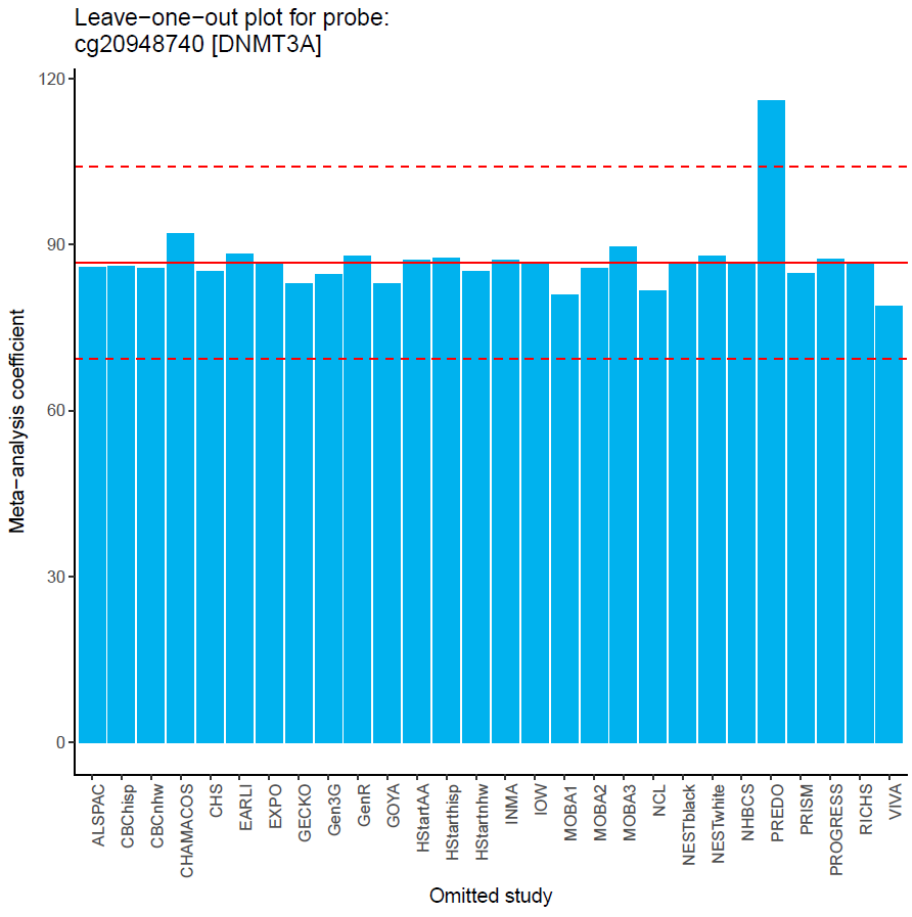
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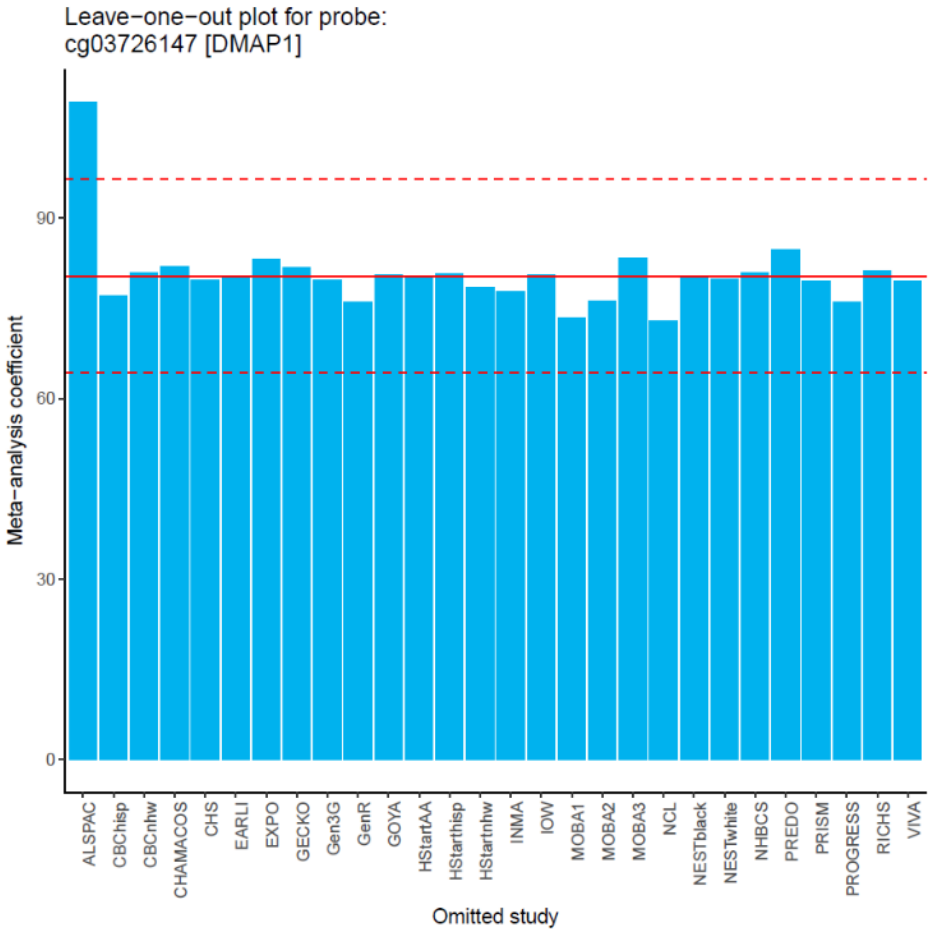
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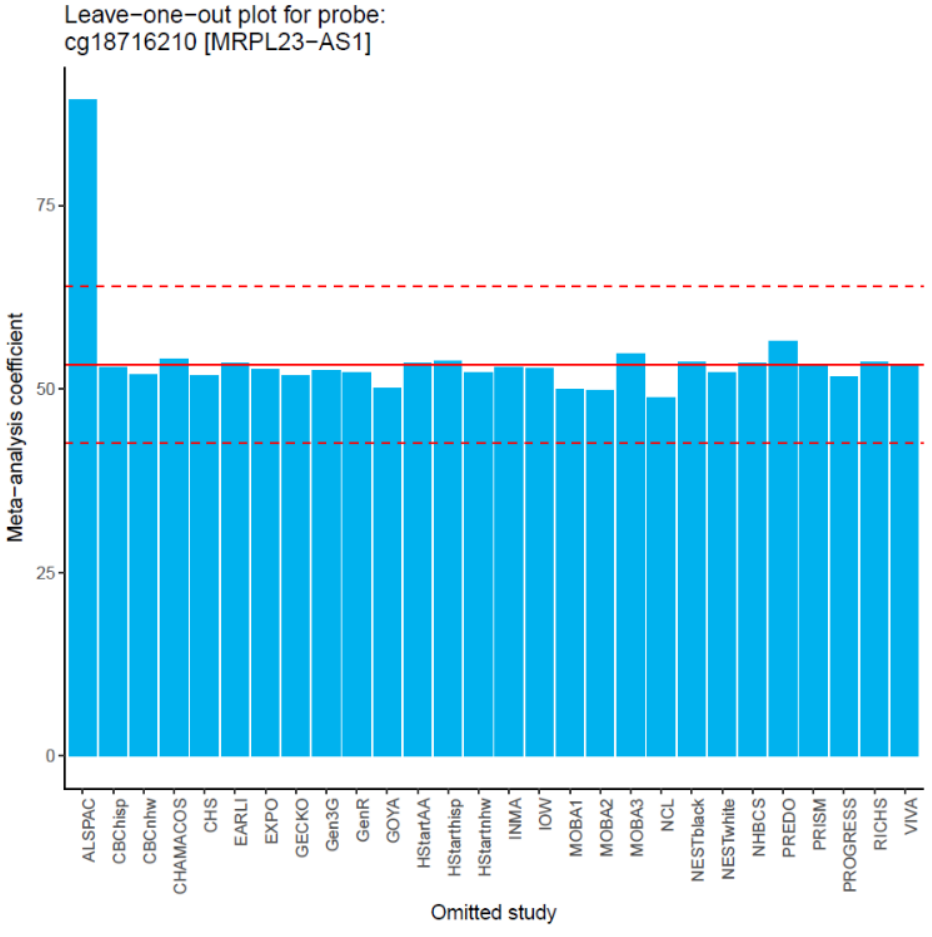
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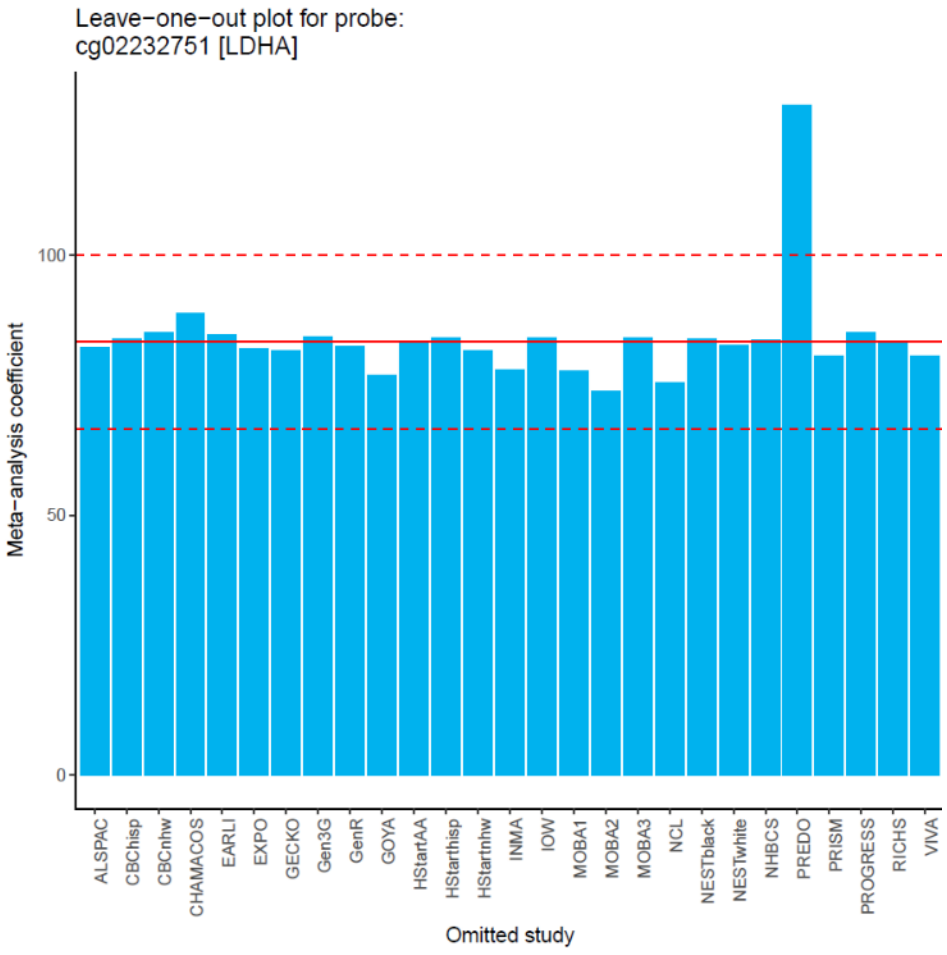
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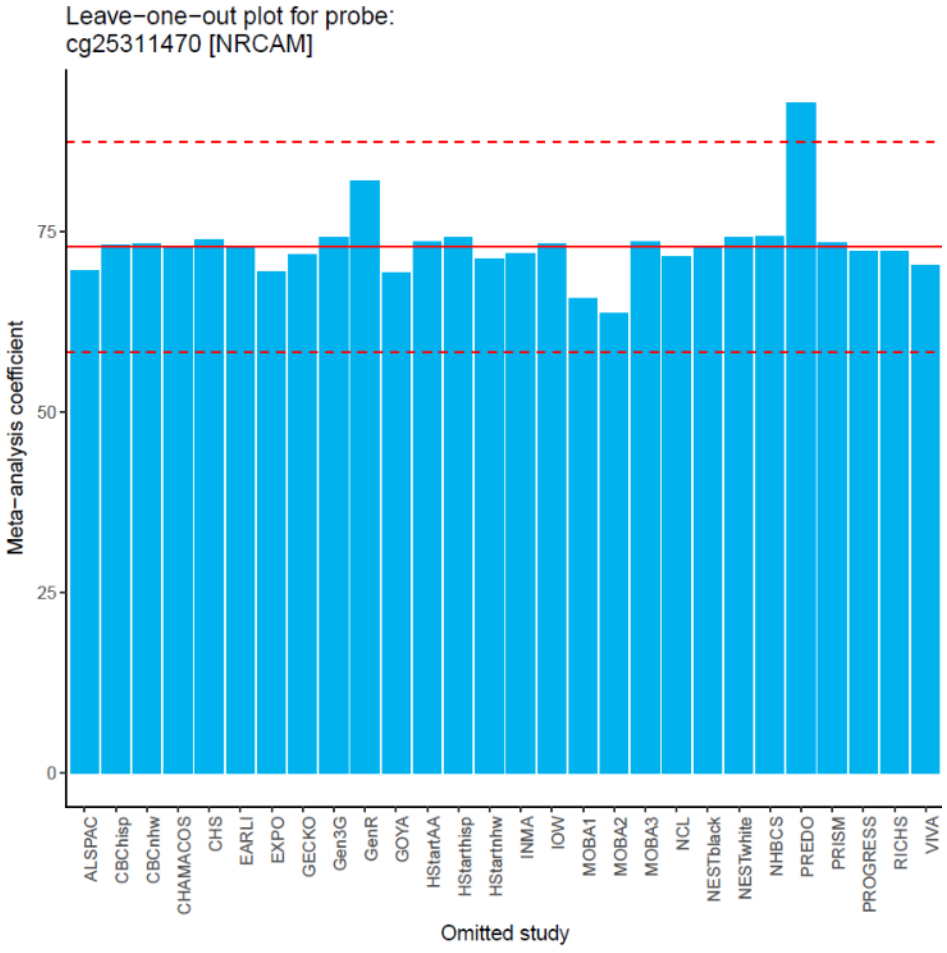
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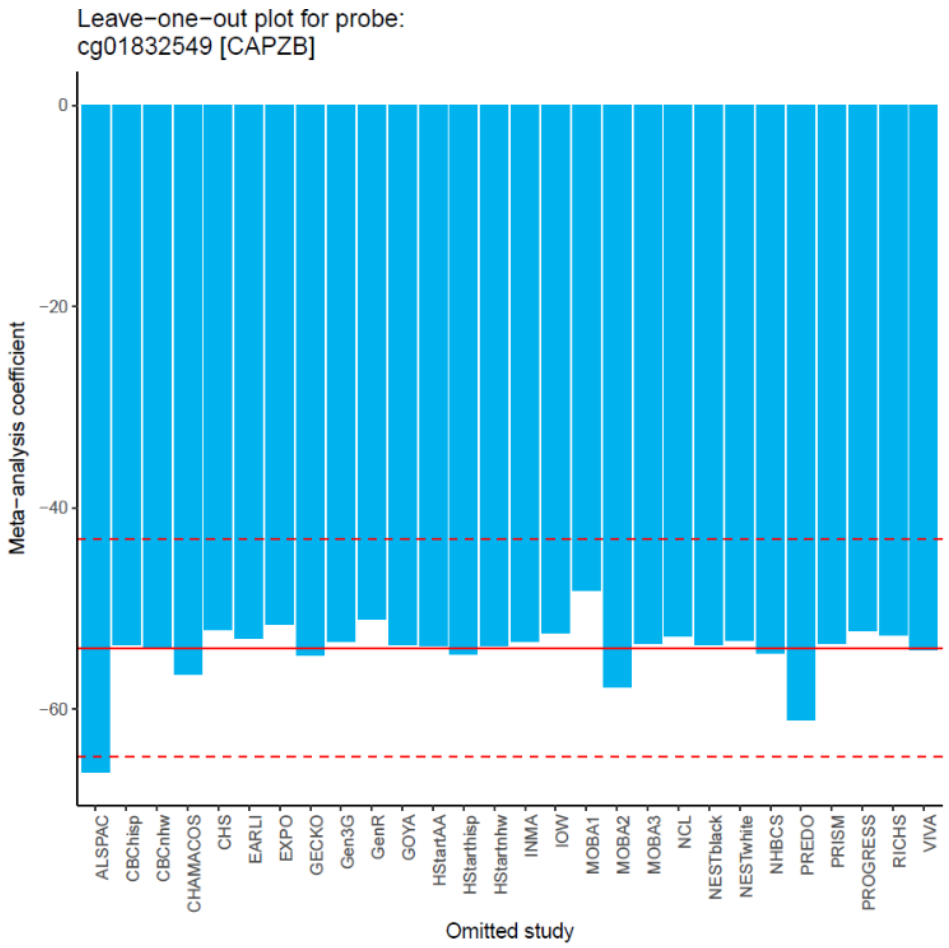
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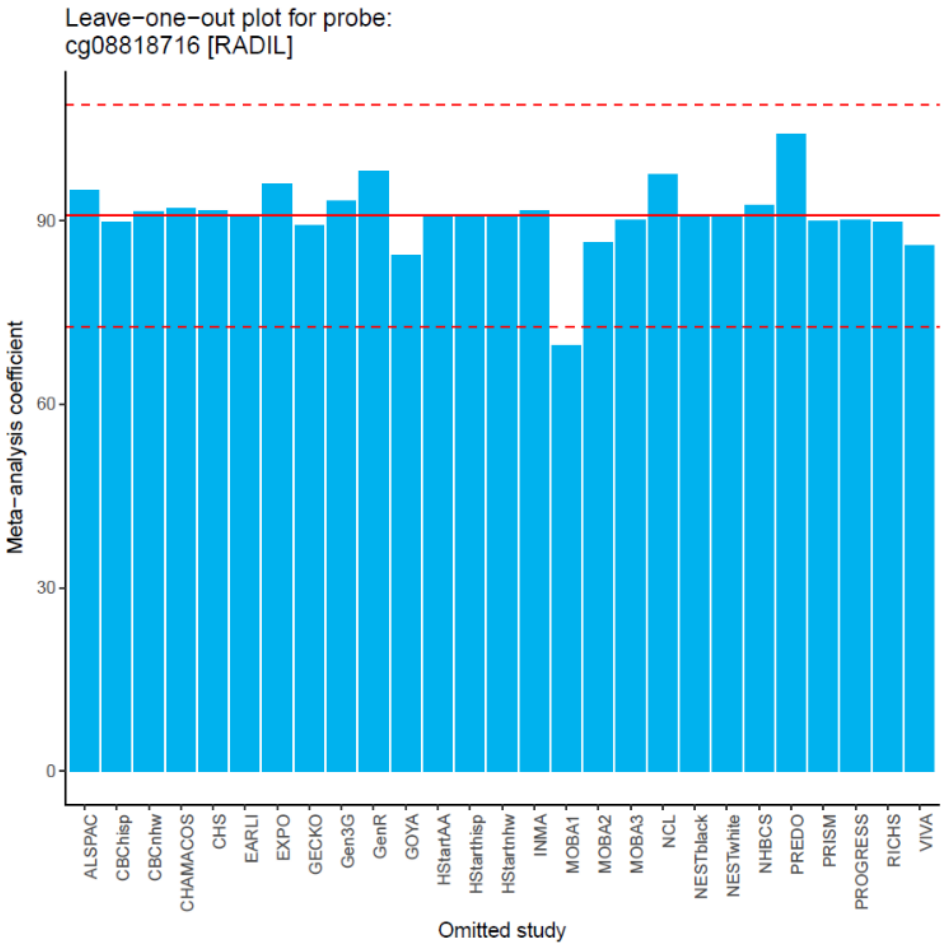
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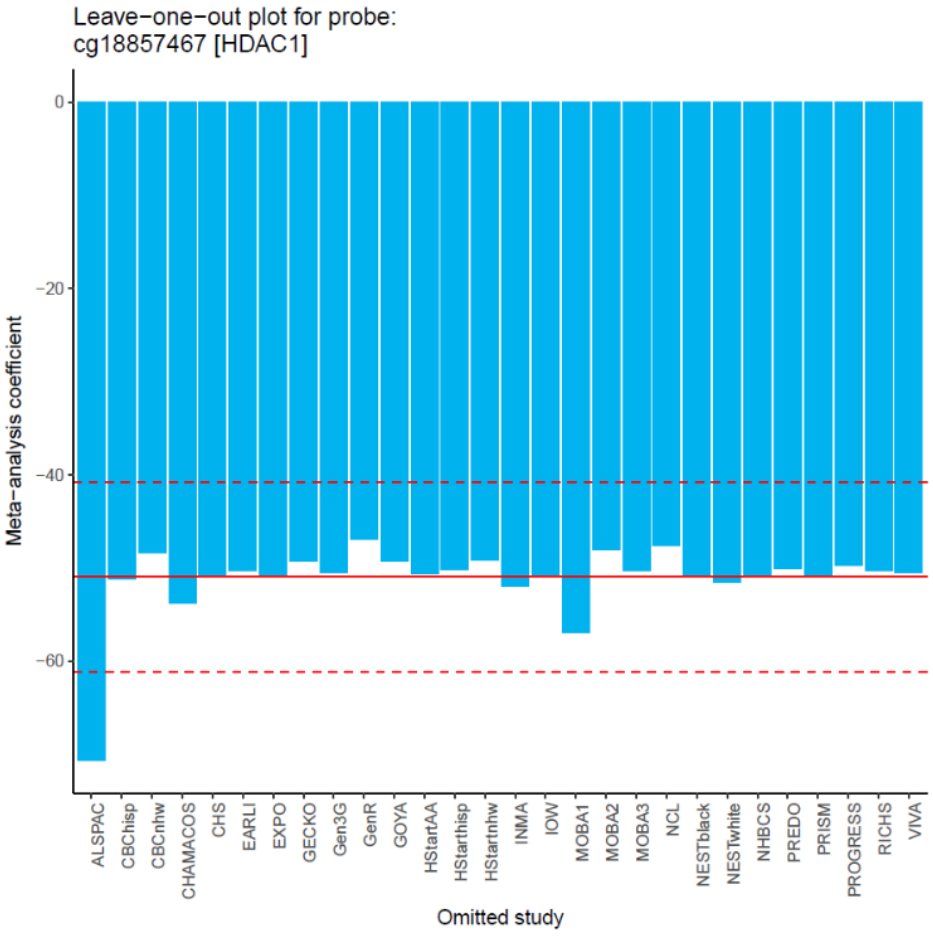
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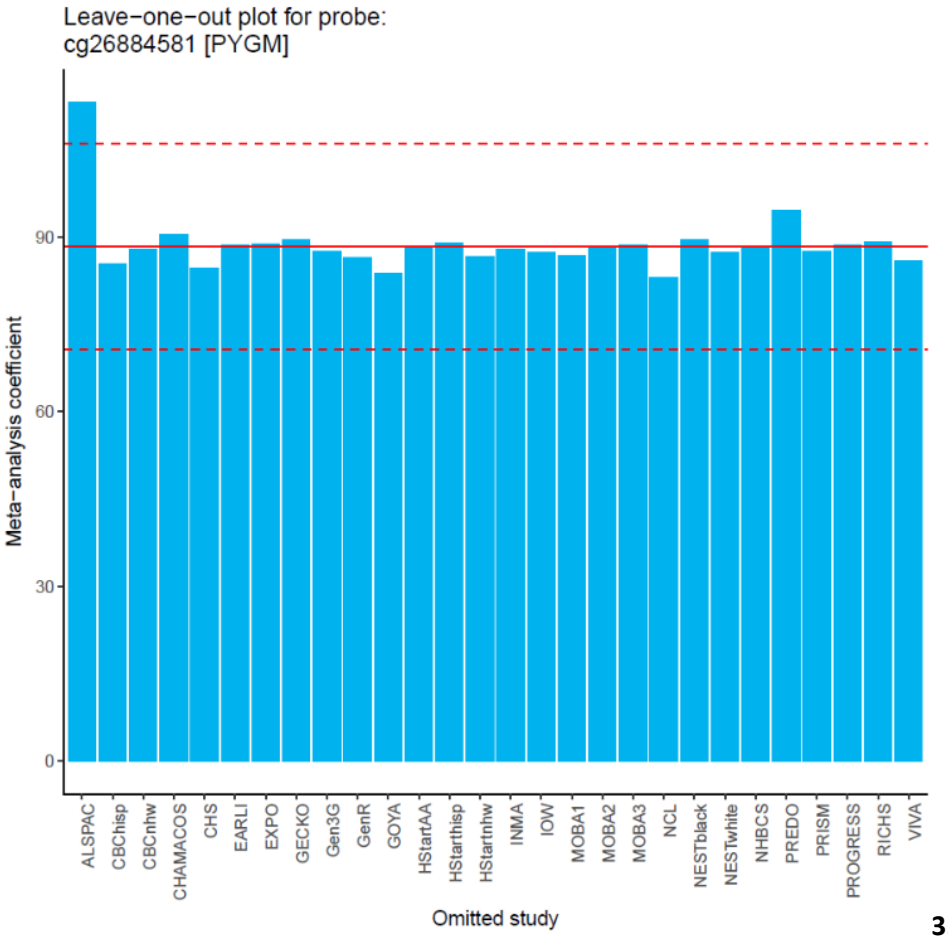
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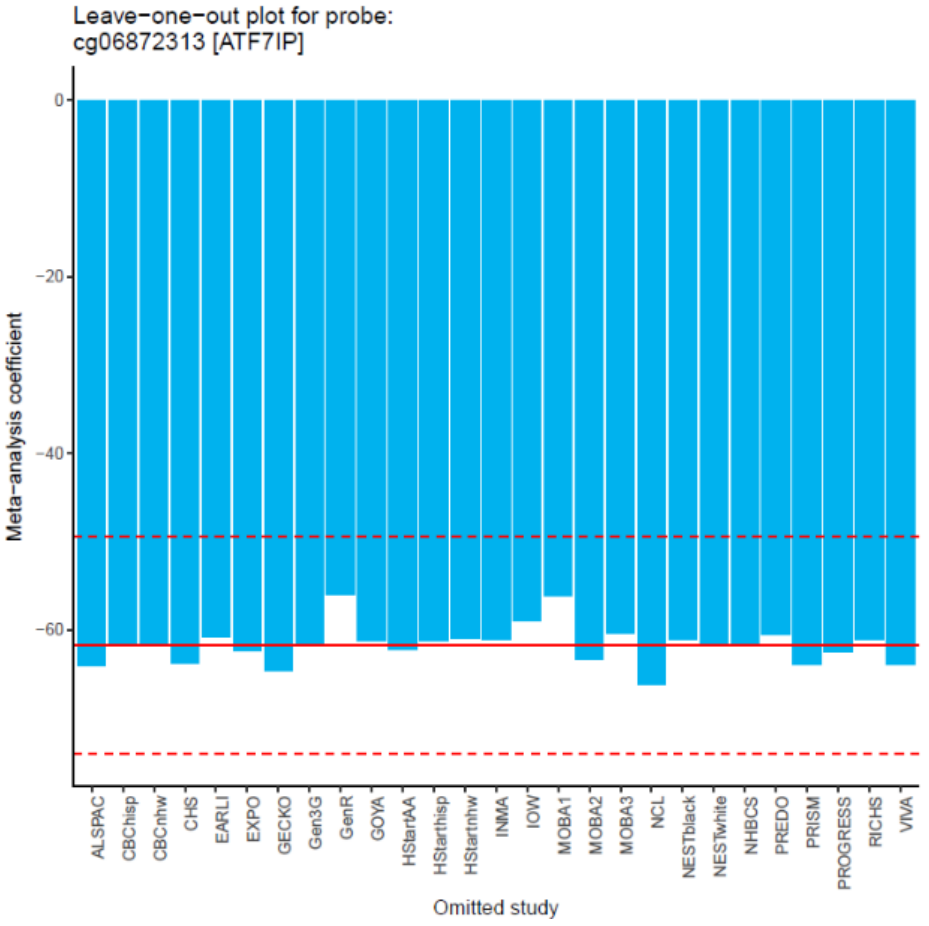
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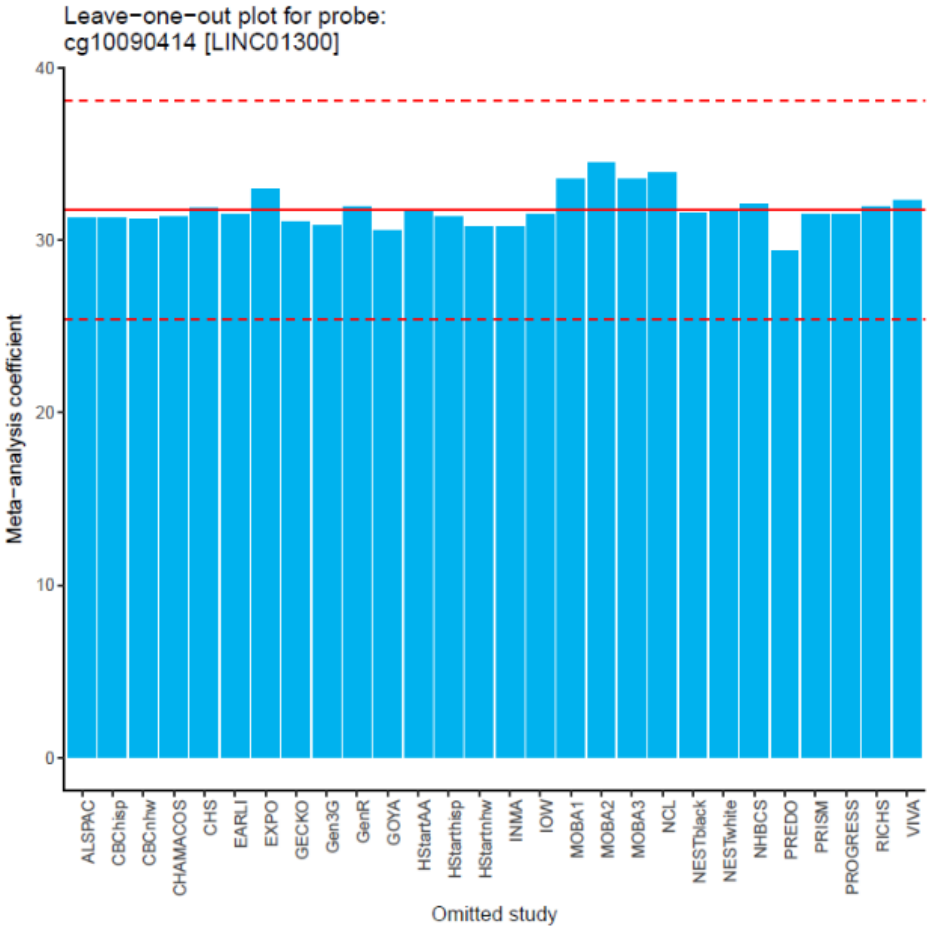
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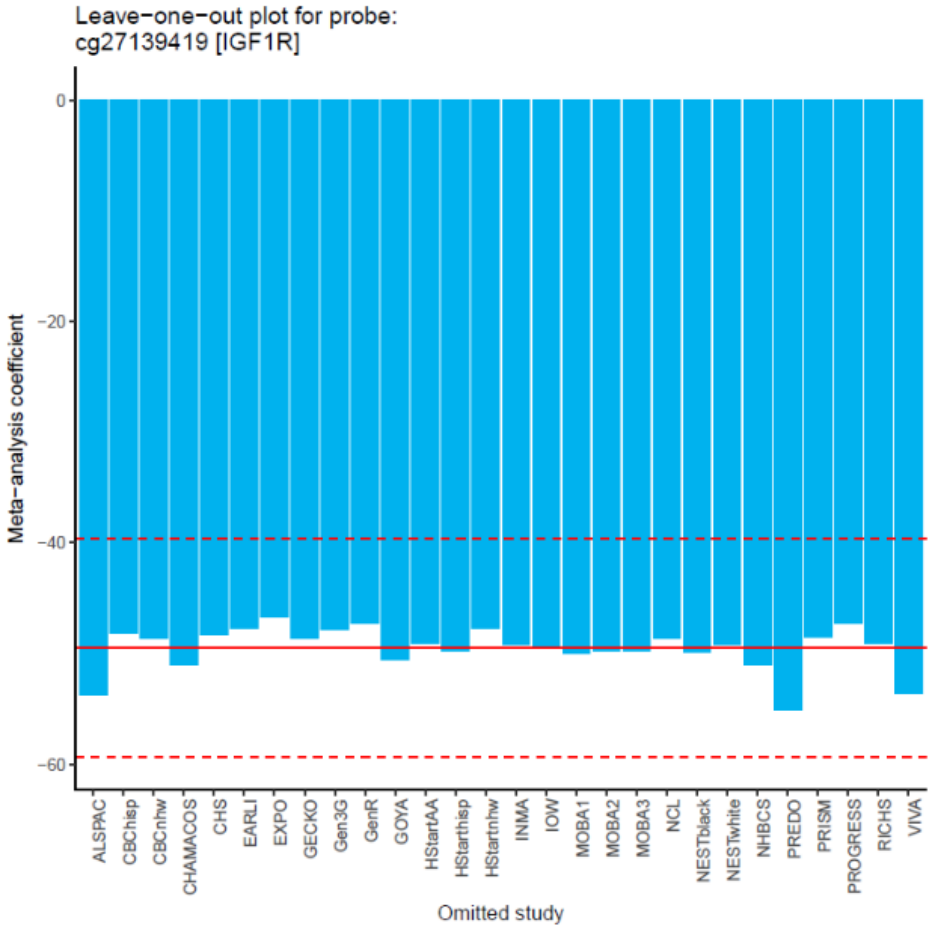
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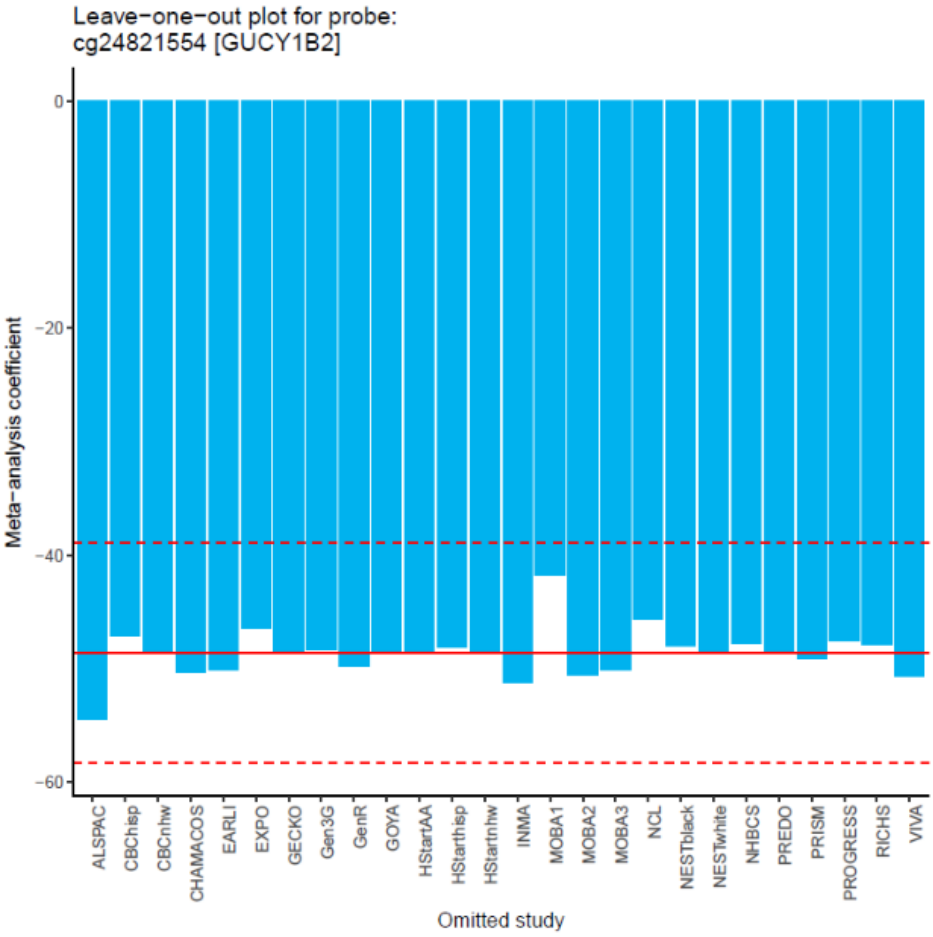
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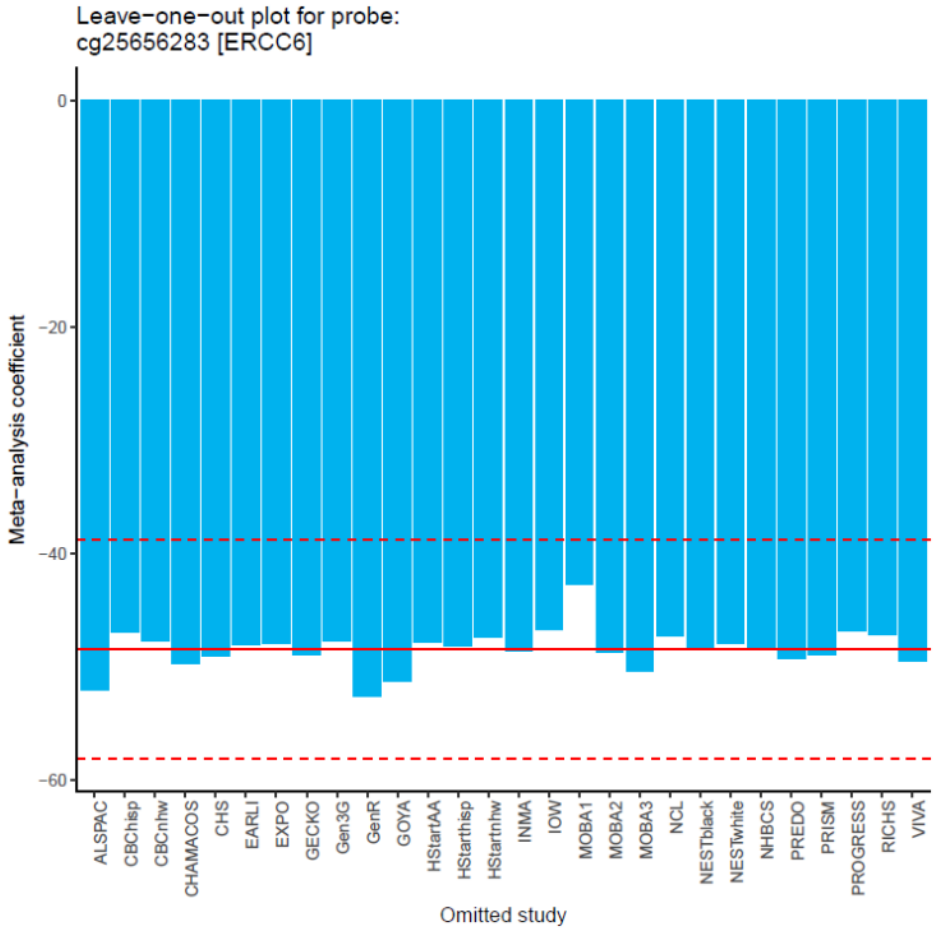
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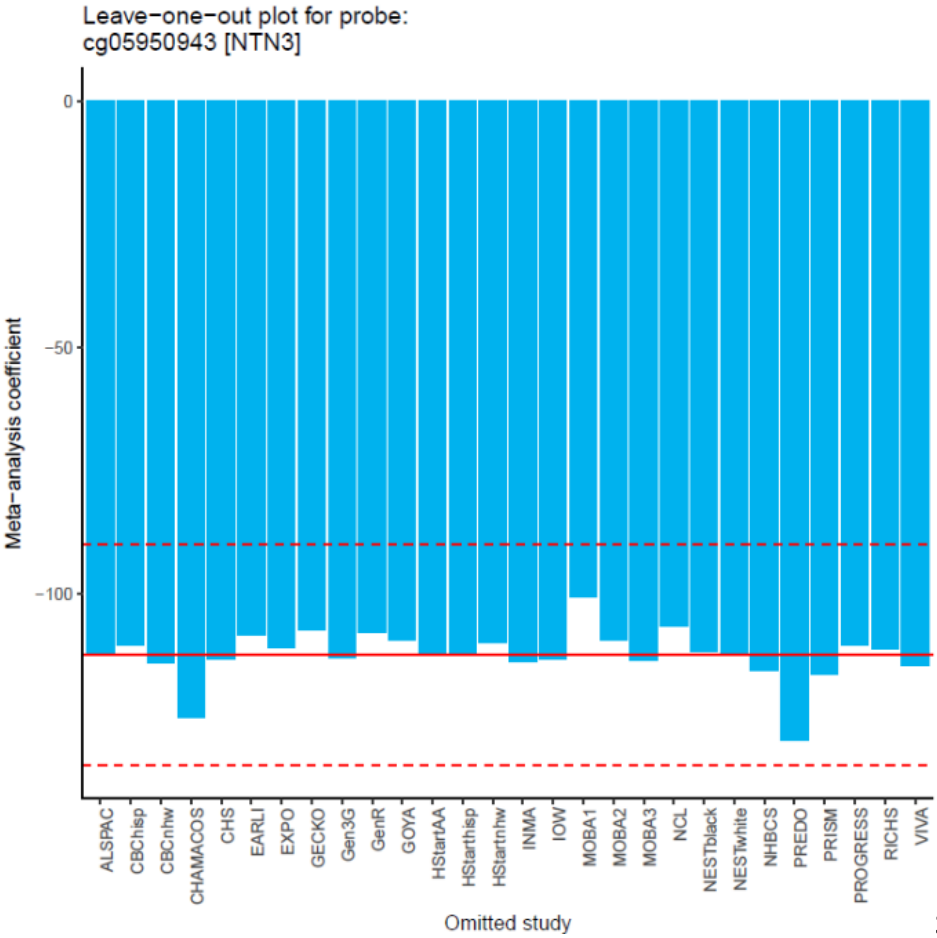
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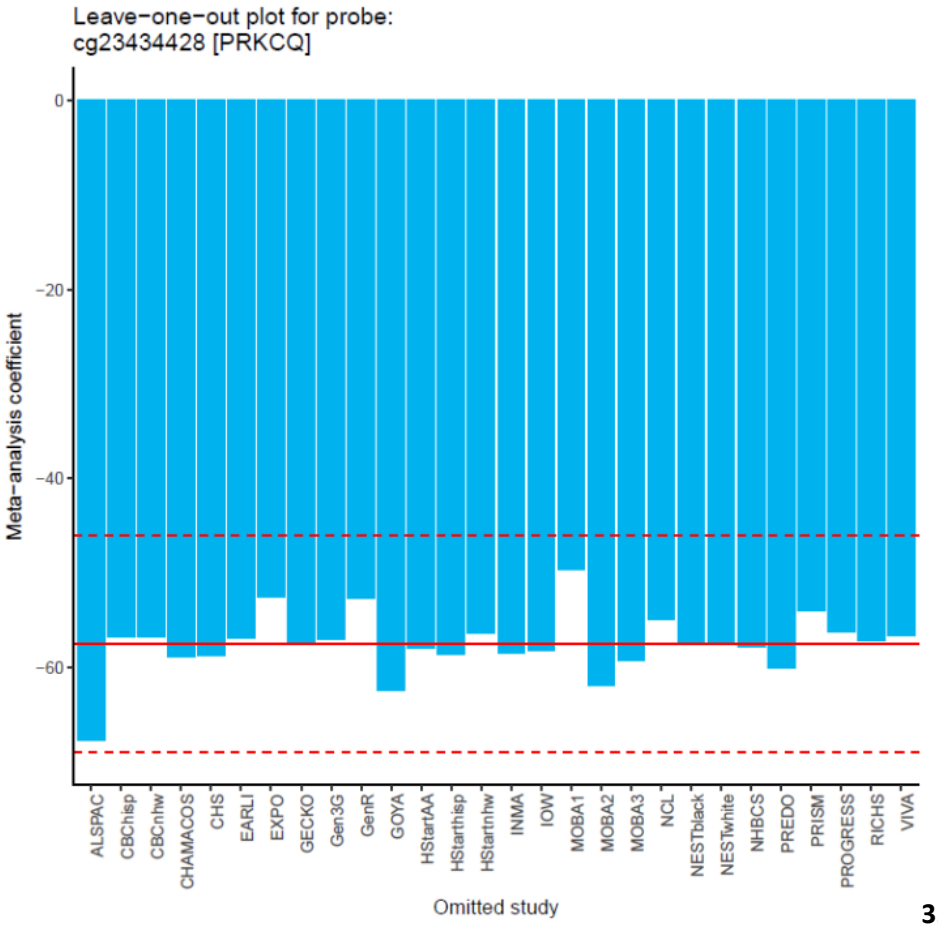
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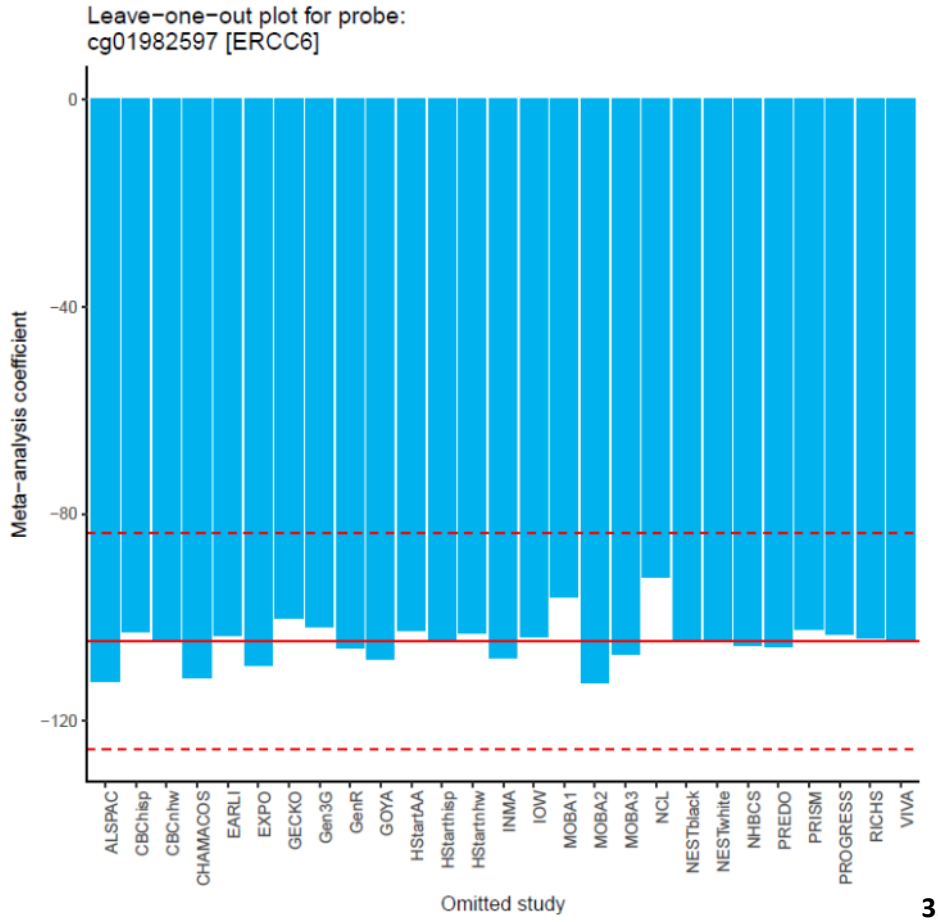
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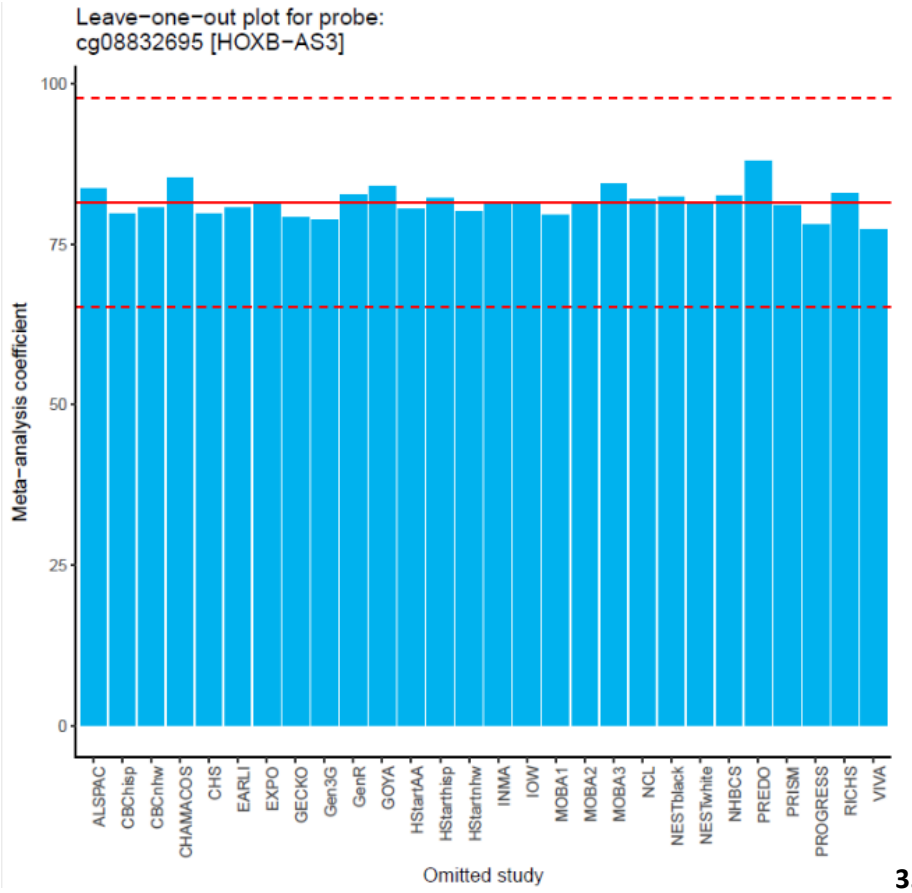

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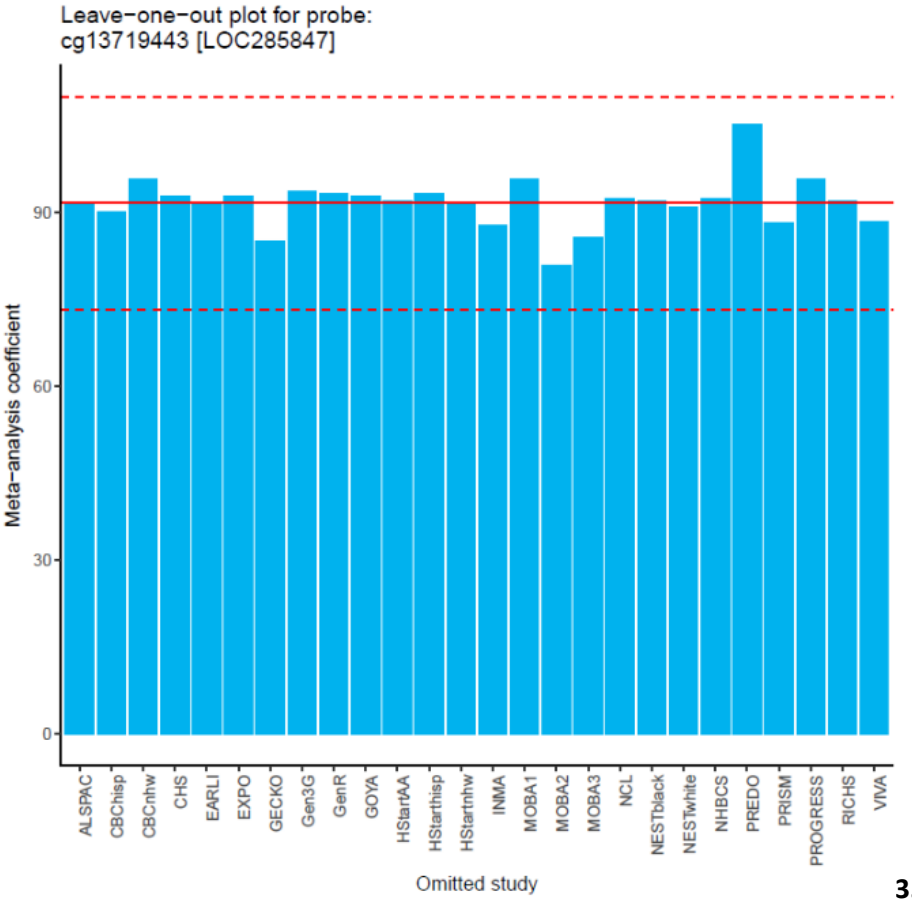
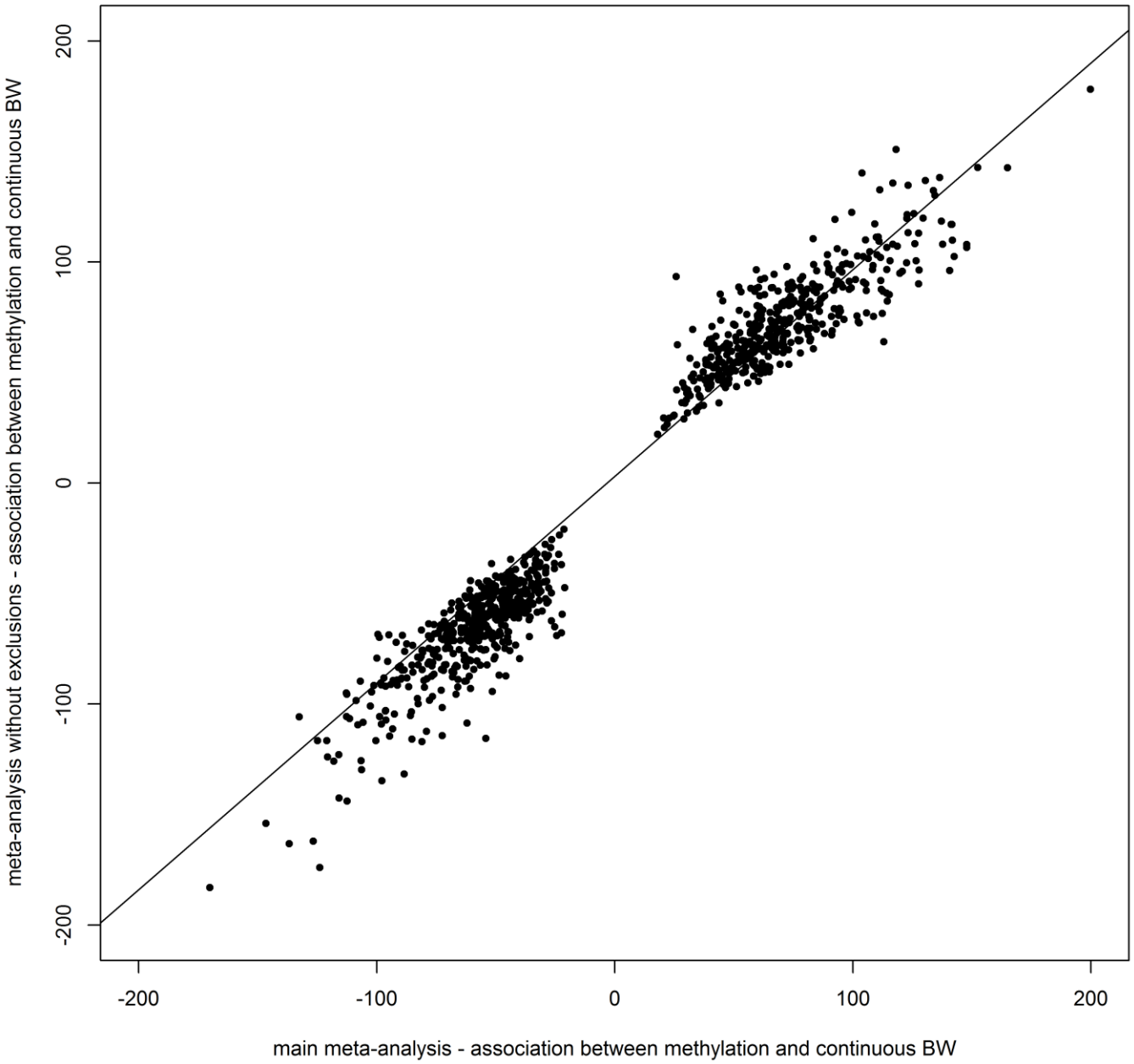


3.20

Supplementary Figures3.1-3.20. Leave-one-out plots for a random 10 (of all 914 birthweight-related) CpGs, showing the association of methylation levels with birthweight as a continuous measure, where removal of one study changed the result by 20% or more (3.1-3.10) and a random 10 where this was not the case (3.11-3.20). The name on the x-axis, representing each bar, is the study that was omitted for that specific meta-analysis.



Supplementary Figure 4. Correlation of the point estimates from the meta-analysis including preterms, pre-eclampsia and diabetes versus main meta-analysis excluding these participants (x-axis)

Correlation of the point estimates for a secondary meta-analysis of the main association of DNA methylation with birthweight including infants born preterm, or to mothers with pre-eclampsia or diabetes (5,414 participants from 9 studies, y-axis) with point estimates for the main meta-analysis with 8,809 participants from 24 studies of mixed ancestry (x-axis).

SUPPLEMENTARY NOTE

Supplementary Funding: study specific and in alphabetical order

ALSPAC

The UK Medical Research Council and the Wellcome Trust (Grant ref: 102215/2/13/2) and the University of Bristol provide core support for ALSPAC. The Accessible Resource for Integrated Epigenomics Studies (ARIES) which generated large scale methylation data was funded by the UK Biotechnology and Biological Sciences Research Council (BB/I025751/1 and BB/I025263/1). Additional epigenetic profiling on the ALSPAC cohort and the contributions of LKK, DAL, CLR, were supported by the UK Medical Research Council Integrative Epidemiology Unit and the University of Bristol (MC_UU_12013_1, MC_UU_12013_2, MC_UU_12013_5 and MC_UU_12013_8). Contributions of authors related to this study and/or additional ALSPAC data were also supported by the Wellcome Trust (WT088806), the United States National Institute of Diabetes and Digestive and Kidney Diseases (R01 DK10324), and the European Research Council under the European Union's Seventh Framework Programme (FP/2007-2013) / ERC Grant Agreement (Grant number 669545; DevelopObese). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. As well as funds from national government and charities, DAL declares that she has received industry support from Roche Diagnostics and Medtronic for work unrelated to that presented here. This project received funding from the European Union's Horizon 2020 research and innovation programme (733206, LIFECYCLE).

BAMSE

BAMSE was supported by The Swedish Research Council, The Swedish Heart-Lung Foundation, MeDALL (Mechanisms of the Development of ALLergy) a collaborative project conducted within the European Union (grant agreement No. 261357), Stockholm County Council (ALF), Swedish foundation for strategic research (SSF) (RBc08-0027), the Strategic Research Programme (SFO) in Epidemiology at Karolinska Institutet and the Swedish Research Council Formas. EM is supported by grants from the Swedish Research Council and the Strategic Research Area Epidemiology at Karolinska Institutet.

CBC

This work was supported by the National Institute of Environmental Health Sciences and the Environmental Protection Agency (grant number: P01ES018172 to C.M., R.R. and J.L.W.); The National Institute of Environmental Health Sciences (grant number: R01ES09137 to C.M., R.R. and J.L.W.); The National Cancer Institute (grant number: R01CA155461 to R.R. and J.L.W.); The Swiss Cancer League (grant number: BIL KLS 3124–02–2013 to S. G.); The Swiss Science National Foundation (grants numbers: P2LAP3_158674 to S.G., P2LAP3_148434 to S.N.); The SICPA Foundation to S.G.; the Sutter-Stottner Foundation to S.G.; a Cancer Center Support Grant (5P30CA082103) to R.R. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the Environmental Protection Agency.

CHAMACOS

CHAMACOS cohort research was supported by grants from the National Institute of Environmental Health Science (NIEHS) [P01 ESO09605, 5UG30D023356, R01ES012503, R01ES021369, R01ES023067, R24ES0285529]; Environmental Protection Agency (RD83273401, RD83171001), and the JPB Foundation. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of NIEHS, EPA, or JPB Foundation.

CHS

This work was supported by NIEHS grants K01ES017801, R01ES022216, and P30ES007048.

EARLI

Funding for the EARLI study was provided by the National Institutes of Health (R01ES016443, PI: Newschaffer) and Autism Speaks. The DNA methylation measures in this study were supported by the National Institutes of Health (R01 ES017646, MPI: Fallin, Feinberg). KB was supported by NIH grants R01ES025531, R01ES025574, and R01 AG055406.

EXPOsOMICS

Piccolipiu: The study was approved and initially funded by the Italian National Centre for Disease Prevention and Control (CCM grant 2010) and by the Italian Ministry of Health (art 12 and 12bis Dl.gs.vo 502/92). The methylation assays were funded by the European Community's Seventh Framework Programme FP7/2007–2013 project EXPOsOMICS (grant no. 308610)

Rhea: The methylation assays were funded by the European Community's Seventh Framework Programme FP7/2007-2013 project EXPOsOMICS (grant no. 308610).

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IOW F1

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Supplementary Methods: study specific and in alphabetical order

Avon Longitudinal Study of Parents and Children (ALSPAC)

Design and study population

ALSPAC is a large, prospective cohort study based in the South West of England. 14,541 pregnant women resident in the previous county of Avon (centred around the city of Bristol), UK with expected dates of delivery 1st April 1991 to 31st December 1992 were recruited. Detailed information has been collected on these women, their partners and their offspring at regular intervals to the present date*(1, 2)*. The study website contains details of all the data that is available through a fully searchable data dictionary (http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/).

Written informed consent has been obtained for all ALSPAC participants. Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees.

Birthweight

Information on birthweight was recorded by healthcare professionals at the time of birth and extracted from birth records. Within ALSPAC, there were no values +/- 5SD from the mean.

Methylation measurements

Cord blood was collected according to standard procedures, spun and frozen at -80˚C. DNA methylation analysis and data pre-processing were performed at the University of Bristol as part of the ARIES project (ariesepigenomics.org.uk)*(3)*. Following extraction, DNA was bisulfite converted using the Zymo EZ DNA MethylationTM kit (Zymo, Irvine, CA). Following conversion, the genome-wide methylation status of over 485,000 CpGs was measured using the Illumina Infinium® HumanMethylation450k BeadChip assay according to the standard protocol. The arrays were scanned using an Illumina iScan and initial quality review was assessed using GenomeStudio (version 2011.1). The level of methylation is expressed as a "Beta" value (β-value), ranging from 0 (no cytosine methylation) to 1 (complete cytosine methylation). Samples from all time-points in ARIES were distributed across slides using a semi-random approach (sampling criteria were in place to ensure that all time-points were represented on each array) to minimize the possibility of confounding by batch effects. Samples failing quality control (average probe detection p-value ≥ 0.01) were repeated. As an additional quality control step genotype probes on the HumanMethylation450k were compared between samples from the same individual and against SNP-chip data to identify and remove any sample mismatches. Data were pre-processed in R (version 3.0.1) with the WateRmelon package according to the subset quantile normalization approach described by Touleimat & Tost in an attempt to reduce the non-biological differences between probes.

We removed probes that had a detection P-value >0.05 for >5% of samples (3034 probes), probes on the X or Y chromosomes and SNPs (rs probes). 471192 probes remained.

Covariates

Data on maternal parity, socio-economic status, smoking, pre-pregnancy body mass index and maternal age at delivery were self-reported by questionnaire during pregnancy. Newborn sex was extracted from birth records and questionnaires after birth. Gestational age at delivery was also extracted from birth records. Obstetric practice and antenatal care at the time means that for most participants gestational age will have been estimated based on the last menstrual period (LMP), supplemented by ultrasound scans and paediatric/obstetric assessment of the newborn at birth. Information on pre-eclampsia and diabetes were extracted from hospital records. For pre-eclampsia all records of any blood pressure and proteinuria across the entire pregnancy were extracted and the International Society for the Study of Hypertension in Pregnancy (ISSHP) criteria applied (Systolic blood pressure >139mmHg OR diastolic blood pressure >89mmHg on at least 2 occasions after 20 weeks of gestation in women who had not previously been diagnosed with hypertension outside of pregnancy, together with proteinuria of 30mg/dl occurring at the same time as the two occasions of elevated blood pressure)*(4)*.

Cell type correction

We used the Reinius-based Houseman method*(5, 6)* with the *estimateCellCounts* function in the Minfi package*(7)* in R*(8)* to estimate relative proportions of six white blood cell subtypes (CD4+ T-lymphocytes, CD8+ T-lymphocytes, NK (natural killer) cells, B-lymphocytes, monocytes and granulocytes).

Batch correction

Ten surrogate variables were generated using the sva package in R and included in models to adjust for technical batch.

Exclusion criteria

For the main analysis we excluded n=38 participants: 19 infants born preterm (<37 weeks), and whose mothers experienced pre-eclampsia (n=12) or diabetes (n=3) during pregnancy, or participants with missing data on either of these. This information was obtained from questionnaires completed by the mothers shortly after delivery and combined with medical birth records.

Children (Barn), Allergy, Milieu, Stockholm, Epidemiology (BAMSE)

Design and study population

BAMSE (Children, Allergy, Milieu, Stockholm, Epidemiology in Swedish) is a prospective population-based cohort study of children recruited at birth and followed during childhood and adolescence. Details of the study design, inclusion criteria, enrolment and data collection are described elsewhere*(9)*. In short, 4,089 children born between 1994 and 1996 in four municipalities of Stockholm County were enrolled. At baseline, when the infant was approximately 2 months of age, parents completed a questionnaire that assessed residential characteristics, as well as socioeconomic and lifestyle factors. When children were 1, 2, 4, 8, 12 and 16 years, the parents completed questionnaires focusing on children's symptoms related to wheezing and allergic diseases, as well as various exposures. The survey response rates were 96%, 94%, 91%, 84%, 82% and 78%, respectively*(10)*. Furthermore, blood was obtained at ages 4, 8 and 16 years from 2,605 (63.7%), 2,470 (60.4%) and 2,547 (62.2%) children, respectively. The baseline and follow-up studies were approved by the Regional Ethical Review Board, Karolinska Institutet, Stockholm, Sweden, and the parents of all participating children provided written informed consent.

Birthweight

Birthweight was assessed by questionnaire at the time of recruitment (at a median age of the children of 2 months), and used as a continuous variable in the analyses*(11)*.

Methylation measurements

For this methylation study, we used data from the 8- and 16-year follow-up. At 8 years, epigenome-wide DNA methylation was measured in 472 Caucasian children and at 16 years, methylation was measured in 269 Caucasian children*(12)*. 500 ng DNA per sample underwent bisulfite conversion using the EZ-96 DNA Methylation kit (Zymo Research Corporation, Irvine, USA). Samples were plated onto 96-well plates in randomized order. Samples were processed with the Illumina Infinium HumanMethylation450 BeadChip (Illumina Inc., San Diego, USA). Quality control of analyzed samples was performed using standardized criteria. Samples were excluded in case of sample call rate <99%, colour balance >3, low staining efficiency, poor extension efficiency, poor hybridization performance, low stripping efficiency after extension and poor bisulfite conversion. We also applied multidimensional scaling (MDS) plot to evaluate gender outliers based on chromosome X data, that produced two separated clusters for male and female. Samples that did not belong to the distinct cluster were removed. Furthermore, we applied median intensity plot for methylated and unmethylated intensity by using the minfi R package (samples below the 10.5 cutoff were excluded). Applying these criteria resulted in exclusion of 8 (8-year DNA) and 2 (16-year DNA) samples, respectively. Probes with a single nucleotide polymorphism in the single base extension site with a frequency of >5% were excluded*(13)*, as were probes with non-optimal binding (non-mapping or mapping multiple times to either the normal or the bisulphite-converted genome), and the probed belonging to chr X and chr Y, resulting in the exclusion of 46,799 and 47,654 probes (8- and 16-year DNA), leaving a total of 438,713 and 437,858 probes, respectively, in the analyses. Furthermore, we implemented "DASEN" recommended from wateRmelon package to do signal correction and normalization*(14)*.

Covariates

Information on maternal BMI in the first trimester and parity was collected from the Birth Registry and parity was categorized into 0 and ≥1*(10)*. Maternal age, sex, maternal smoking status and maternal socio-economic status information were collected from a questionnaire administered at enrolment. Maternal socio economic was categorized into blue collar worker, white collar worker and other students, unemployed, housewives. Gestational age was from both the Birth Registry and a questionnaire administered at enrolment. Current age and current BMI was collected from the 8- and 16-year questionnaires, respectively. Ever doctor's diagnosis of asthma was considered to be a selection factor in the 8-year data, but not at 16 years.

Cell type correction

We used the Reinius-based Houseman method*(5, 6)* with the estimateCellCounts function in the Minfi package*(7)* in R*(15)* to estimate relative proportions of six white blood cell subtypes (CD4+ T-lymphocytes, CD8+ T-lymphocytes, NK (natural killer) cells, B-lymphocytes, monocytes and granulocytes).

Batch correction

The covariate batch was also accounted for in the models, based on the bisulfite treatment date at 8 years. At 16 years, the empirical Bayes method via ComBat was applied for batch correction based on sample plate and sentrix position using the sva package in R*(16)* (Johnson et al. 2007)

Exclusion criteria

We applied the following exclusion criteria: multiple births (i.e. singleton only analysis), preterm births (i.e. ≥37 weeks only), maternal diabetes, pre-eclampsia.

California Birth Cohort (CBC)

Design and study population

The California Department of Public Health maintains a repository of all neonatal birth bloods as blood dried on a filter paper (Guthrie card), which we term the California Birth Cohort (CBC). These are available for qualified researchers to perform specified health research as monitored by local and State level institutional review boards; all work here was performed under ethics approval at the State of California and University of California. Our current research project using CBC resources is a case-control study of childhood leukemia, the California Childhood Leukemia Study (CCLS) which identifies children with leukemia and matched

controls (birthdate, gender, and ethnicity) from around the State of California*(17)*. Control subjects were chosen as birthdate, gender, and ethnic matches to leukemia cases. Cases included all consecutive cases at 13 California hospitals which treat leukemia.

Birthweight

Birthweight data was collected by obstetricians in the delivery room and recorded on the birth certificate, which was used for the current analysis. We double checked and did not observe birthweight values +/- 5 SD from the mean. For the main analysis model, we treated birthweight as a continuous trait in grams and for the dichotomous model we categorised birthweight in high (>4000g) versus normal (2500-4000g) birthweight.

Methylation measurements

Approximately 300-500 ng of high molecular weight DNA was extracted from a 1/4 section of a 1.5 cm2 archived neonatal DBS (stored at -20°C from the time of birth) using Qiagen blood card extraction protocol and bisulfite treated using the EZ DNA Methylation-Direct™ Kit (Zymo). Genome-wide DNA methylation was then measured in these bisulfite-converted DNA samples using Illumina(C) Infinium HumanMethylation450 BeadChip arrays. CpGs with detection p-values > 0.01 were defined as bad CpGs and discarded. CpGs with >15% of absence of information (i.e. >15% of total samples) were totally excluded from the analysis. A total of 540 CpGs were excluded. Samples with >15% of bad CpGs (of the 450K loci) were also excluded from the analysis. The DNA methylation data preprocessing consisted of functional normalization according to Fortin et al.*(18)* to control for batch and position effects. Additional correction for probe types was accomplished with BMIQ normalization*(19)*. Joo et al. have demonstrated that DNA methylation measured by the HM450k array on archived dried blood spots is fully correlated with DNA methylation measured by the same platform on same individuals' matched frozen buffy coats (correlation coefficient = 0.99), therefore proving that this material is suitable for DNA methylation analyses*(20)*.

Covariates

Gestational age, gender, trichotomous annual household income <=$44,999/<=$74,999/>=$75,000 as maternal social class, dichotomous maternal smoking status, maternal age and maternal BMI, parity were used as covariates in the models. Models were stratified by ethnicity group (Hispanic and Caucasian).

Cell type correction

Estimation of six different white blood cell types (CD8+ T and CD4+ T lymphocytes, CD56+ natural killer cells, CD19+ B cells, CD14+ monocytes, and granulocytes) by Houseman method*(6)* was performed using the default implementation of the *estimateCellCounts* function in the minfi package*(7)*.

Batch correction

Analyses were adjusted for batch by including the Beadchip ID.

Exclusion criteria

Information on maternal diabetes and pre-eclampsia was not available, thus we were not able to filter data based on these criteria. For this study we only included control participants, participants with leukemia were excluded.

The Center for the Health Assessment of Mothers and Children of Salinas study (CHAMACOS)

Design and study population

The Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) study is a longitudinal birth cohort study of the effects of exposure to pesticides and environmental chemicals on the health and development of Mexican-American children living in the agricultural region of Salinas Valley, CA. Detailed description of the CHAMACOS cohort has previously been published*(21, 22)*. Briefly, 601 pregnant women were enrolled in 1999-2000 at community clinics and 527 liveborn singletons were born. Follow up visits occurred at regular intervals throughout childhood. Study protocols were approved by the University of California, Berkeley Committee for Protection of Human Subjects and written informed consent was obtained from all mothers; oral assent was obtained from children beginning at age 7, and written assent at age 12.

Birthweight

Infant birthweight (grams) was collected from medical records and abstracted by a registered nurse. We did not observe birthweight values +/- 5 SD from the mean.

Methylation measurements

DNA methylation was measured in DNA isolated from the cord blood of 378 CHAMACOS newborns, 244 nine year samples, and 60 twelve year samples by Illumina Infinium HumanMethylation450 (450K) BeadChips. DNA samples were bisulfite converted using Zymo Bisulfite Conversion Kits (Zymo Research, Irvine, CA), whole genome amplified, enzymatically fragmented, purified, and applied to the 450K BeadChips (Illumina, San Diego, CA) according to manufacturer protocol. 450K BeadChips were handled by robotics and analyzed using the Illumina Hi-Scan system. DNA methylation was measured at 485,512 CpGs.

Probe signal intensities were extracted by Illumina GenomeStudio software (version XXV2011.1, Methylation Module 1.9) methylation module and background subtracted. QA/QC was performed systematically by assessment of assay repeatability batch effects using 38 technical replicates, and data quality established as previously described [3]. Quality was also ensured by only retaining samples where 95 % of sites assayed had detection p> 0.01. The same threshold (95% detection at p>0.01) was imposed to CpGs as well (n= 460 removed). Sites with annotated probe SNPs and with common SNPs (minor allele frequency >5%) within 50bp of the target identified in the MXL (Mexican ancestry in Los Angeles, California) HapMap population were excluded from analysis (n=49,748). This left a total of 435,369 CpGs in the analysis. Color channel bias, batch effects and difference in Infinium chemistry were minimized by application of ASMN algorithm*(23)*, followed by BMIQ normalization*(19)*.

Covariates

Maternal age, parity and education were assessed by participant interview at baseline visit (~13 weeks gestation). Maternal education was used as a proxy for maternal social class. Maternal education was treated as categorical, with two levels: less than a completed high school education, or having completed high school education or beyond. Maternal pre-pregnancy BMI was calculated using self-reported pre-pregnancy weight when interviewed at enrolment and measured height. Information on maternal smoking status was obtained through participant interview at baseline (~13 weeks gestation), follow up interview (~26 weeks gestation), and delivery.

Cell type correction

We used the Reinius-based Houseman method*(5, 6)* with the *estimateCellCounts* function in the Minfi package*(7)* in R*(8)* to estimate relative proportions of six white blood cell subtypes (CD4+ T-lymphocytes, CD8+ T-lymphocytes, NK (natural killer) cells, B-lymphocytes, monocytes and granulocytes).

Batch correction

Analysis was also adjusted for batch effects by including 450K plate (n=10) as additional covariates.

Exclusion criteria

For the main analysis we excluded n=85 unique participants after overlap: 31 infants born preterm (<37 weeks), and whose mothers experienced pre-eclampsia (n=36) or diabetes (n=80) during pregnancy. This information was obtained from questionnaires completed by the mothers shortly after delivery and combined with medical birth records.

Children's Health Study (CHS)

Design and study population

The Children's Health Study (CHS) is a population-based prospective cohort study from age 5 onwards in Southern California, which has been described in detail elsewhere*(24)*. The study protocol was approved by the University of Southern California Institutional Review Board and informed, written consent and assent were provided by the parents and children respectively. A total of 5341 children were recruited, all of whom were born between 1995 and 1997 and are currently being followed until age 18.

Birthweight

Newborn birthweight was obtained from California birth certificates. We did not observe birthweight values +/- 5 SD from the mean.

Methylation measurements

Based on the availability of newborn bloodspots archived by the state of California, a subset of 273 children was selected for a sub-study in which epigenome-wide DNA methylation was assessed in newborn bloodspots using the Infinium HumanMethylation450 BeadChip (HM450). Laboratory personnel performing DNA methylation analysis were blinded to study subject information. DNA was extracted from whole blood cells using the QiaAmp DNA blood kit (Qiagen Inc, Valencia, CA) and stored at -80 degrees Celcius. 700-1000ng of genomic DNA from each sample was treated with bisulfite using the EZ-96 DNA Methylation Kit™ (Zymo Research, Irvine, CA, USA), according to the manufacturer's recommended protocol and eluted in 18 μl. Chips were analysed in three batches over a period of a couple of months. The results of the Infinium HumanMethylation450 BeadChip (HM450) were compiled for each locus and were reported as beta (β) values. A normal-exponential background correction with dye bias correction was applied to the raw intensities at the array level to reduce background noise*(25)*. We then normalized each sample's methylation values to have the same quantiles to address sample to sample variability*(26)*. CpG loci on the HM450 array were removed from analyses if they were on the X and Y chromosomes, or if they contained SNPs, deletions, repeats, or if they have more than 10% missing values, leaving a total of 383,857 probes for analysis. Beta values were considered as outliers and were removed if they fall below Quartile 1-3×IQR or above Quartile 3+3×IQR.

Covariates

Gestational age at birth, newborn gender, maternal age at birth and maternal parity were obtained from California birth certificates. Data on maternal social class and maternal smoking status during pregnancy were obtained from parent-completed questionnaires at study entry when the subjects were around 6 years old. Ancestry was assessed from CHS genome-wide genotypic

data using the program STRUCTURE from a set of ancestral informative markers that were scaled to represent the proportion of African American, Asian, Native American and white admixture*(27)*. The CHS sample consisted of 55.3% Hispanic white, 32.7% non-Hispanic white, and 12.1% Asian/other acenstries.

Cell type correction
Six white blood cell subtypes were estimated using the Reinius-based Houseman method*(5, 6)*. Estimated cell subpopulations (CD8+ T-lymphocytes, CD4+ T-lymphocytes, natural killer cells, B-lymphocytes, monocytes and granulocytes) were subsequently included as linear predictors in regression models.

Batch correction
We additionally corrected the analyses for batch effect by including the Illumina Infinium HumanMethylation450 BeadChip plate number (n=3).

Exclusion criteria
For the main analysis we excluded n=24 participants: 12 infants born preterm (<37 weeks), and whose mothers experienced pre-eclampsia (n=4) or diabetes (n=5) during pregnancy. This information was obtained from California birth certificates. Additionally, we excluded subjects with missing data on birthweight or any of the other covariates in the model (gestational age, gender, maternal education level, maternal smoking status, maternal age, parity, methylation plate, estimated cell proportions and ancestry), leaving 199 subjects in the analysis.

Early Autism Risk Longitudinal Investigation (EARLI)

Design and study sample
The Early Autism Risk Longitudinal Investigation (EARLI) is an enriched risk prospective pregnancy cohort to study autism etiology*(28)*. The EARLI study was reviewed and approved by Human Subjects Institutional Review Boards (IRBs) from each of the four study sites (Johns Hopkins University, Drexel University, University of California Davis, and Kaiser Permanente Northern California). This longitudinal study recruited mothers of confirmed autism spectrum disorder (ASD) children who were early in a subsequent pregnancy or were trying to become pregnant. There were 232 mothers with a subsequent sibling born through this study. All children were born between November 2009 and March 2012. Demographics, maternal behaviors, food frequency, medical history were all collected via questionnaire. Biosamples and house samples were collected during pregnancy, at birth, and during development. EARLI consisted of 49.6% European, 13.0% Hispanic, 7.6% African and 29.8% other ancestries.

Birthweight
Birthweight was obtained from medical records abstraction. No birthweight values met the exclusion criteria (+/- 5SD).

Methylation measures
Biospecimens including cord blood and placenta were collected and archived at 213 births. Cord blood DNA was extracted using the DNA Midi kit (Qiagen, Valencia, CA) and samples were bisulfite treated and cleaned using the EZ DNA methylation gold kit (Zymo Research, Irvine, CA). DNA was plated randomly and assayed on the Infinium HumanMethylation450 BeadChip (Illumina, San Diego, CA) at the Johns Hopkins SNP Center, a shared lab and informatics operation with the Center for Inherited Disease Research (Johns Hopkins University). Methylation control gradients and between-plate repeated tissue controls were used. We used the minfi library (version 1.18.2) in R (version 3.3) to process raw Illumina image files into noob background corrected methylation values*(18, 25)*. Probes with failed detection P-value (>0.05) in >10% of samples were removed (n=508). Samples with discordant methylation predicted sex and observed sex were removed (n=2) as were samples that appeared as outliers on the first principal component of methylation data across the genome prior to normalization (n=2). Of the remaining 175 cord blood 450k samples, birthweight and complete covariate status were available on 131 participants. This resulted in 2 low birthweight, 16 high birthweight, and 113 normal birthweight children. We excluded control probes, cross-reactive probes*(13)* and probes that did not meet our criteria of a detection p value of the samples, resulting in 455,698 remaining CpGs.

Covariates
Models were adjusted for self-reported maternal education, maternal age, and smoking level. We also adjusted for newborn sex and gestational age, which were obtained from medical records. We further adjusted for ancestry principal components 1 and 2, estimated cell counts (n=6) and hybridization date. By selection, maternal parity for the study was >1 and we did not adjust models for parity.

Cell type correction
Proportion of cell types was estimated using the estimateCellCounts function in the minfi package*(29)* using the Houseman method*(6)* with the adult reference panel*(5)*. Cell types estimated were CD4+ T-lymphocytes, CD8+ T-lymphocytes, NK (natural killer) cells, B-lymphocytes, monocytes and granulocytes.

Batch correction
We adjusted noob normalized data for batch effect by hybridization date using ComBat in the sva package (version 3.9.1)*(30)*.

Exclusion criteria
For the main analysis we excluded n=44 participants: 34 missing birthweight data, 2 sustained smokers, 1 missing gestational age data. No infants were born preterm (<37 weeks). This information was obtained from questionnaires completed by the mothers throughout pregnancy and shortly after delivery and combined with medical birth records.

EXPOsOMICS

Design and study population
Within the EXPOsOMICS collaborative European project, a combination of three population-based birth cohorts ENVIRONAGE, Rhea and Piccolipiu was established to conduct DNA methylation analyses*(31)*. The phenotypic variables were harmonized across the three cohorts, and their biospecimen were randomized on the DNA methylation arrays.

Piccolipiu: Piccolipiu is a multicentric Italian birth cohort that recruited 3338 newborns and their mothers in 5 centres: Turin, Trieste, Florence, Viareggio and Rome between 2011 and 2015. Details about the study protocol have been published elsewhere*(32)*. Families were contacted 6, 12, 24 and 48 months after delivery to collect follow-up information using questionnaires, and children underwent a medical examination at 4 years of age. Cord blood was collected and stored in a centralized biobank. Ethical approvals have been obtained from the Ethics committees of the Local Health Unit Roma E (management centre), of the Istituto Superiore di Sanità (National Institute of Public Health) and of each local centre. Parents provided written informed consent. A sample of 99 children from the Turin centre -who were resident in Turin, with growth data at birth and until at least 2 years of age and full availability of cord blood samples- was included in the EXPOsOMICS Children Studies.

Rhea: The mother-child "Rhea" study in Crete is a prospective cohort examining a population sample of pregnant women and their children, at the prefecture of Heraklion (n = 1500)*(33)*. Ethical approval was obtained and informed consent was given. The study aims are to evaluate a) nutritional, environmental, biological and psychosocial exposures in the prenatal period and in early childhood, b) the association of these exposures with the development of the foetus and the child, c) mother's health during and after pregnancy, and d) genetic susceptibility and the interactions between genetic and environmental factors affecting child health. A set of 100 children from the RHEA-cohort is included in the EXPOsOMICS children studies, for which data on cord blood DNA-methylation is available.

ENVIRonAGE: The ENVIRonAGE (ENVIRonmental influence ON AGEing in early life) cohort includes 1500 mother-infant pairs (Belgium) and further recruitment is ongoing*(34)*. Ethical approval was obtained and written informed consent was given by the parents. Data include mothers' lifestyle and socio-economic status, gestational history, measurements including the new-borns' blood pressure (all healthy), bio-banked placental tissue and cord blood including RNA/DNA, toxic metals in cord blood and placenta, and in utero and early life exposure to fine particulates and NO2 using a spatial temporal interpolation method. A set of 200 children from the ENVIRonAGE-cohort is included in the EXPOsOMICS children studies, for which data on cord blood DNA methylation is available.

Birthweight
Piccolipiu: Newborn's birthweight was abstracted from obstetric records. Outliers were identified through inspection of the joint distribution of birthweight and gestational duration. We did not observe birthweight values +/- 5 SD from the mean. For the main analysis model, we treated birthweight as a continuous trait in grams, and, for the dichotomous model, we categorised birthweight in high (>4000g) versus normal (2500-4000g) birthweight.

Rhea: Newborn's birthweight was abstracted from obstetric records. Outliers were identified through inspection of the joint distribution of birthweight and gestational duration. We did not observe birthweight values +/- 5 SD from the mean. For the main analysis model, we treated birthweight as a continuous trait in grams, and, for the dichotomous model, we categorised birthweight in high (>4000g) versus normal (2500-4000g) birthweight.

ENVIRonAGE: Newborn's birthweight was abstracted from obstetric records. Outliers were identified through inspection of the joint distribution of birthweight and gestational duration. We did not observe birthweight values +/- 5 SD from the mean. For the main analysis model, we treated birthweight as a continuous trait in grams, and, for the dichotomous model, we categorised birthweight in high (>4000g) versus normal (2500-4000g) birthweight.

Methylation measurements
The size of the selected sample set from EXPOsOMICS birth cohorts is 399 newborns: Environage (n=200), Picolli+ (n=99) and RHEA (n=100). From these newborns, cord blood DNA was used for the epigenome-wide DNA methylation analyses, and the samples were randomized across the three cohorts before the subsequent processing steps. Bisulfite conversion (600 ng DNA/sample) was performed using the EZ-96 DNA methylation kit (Zymo research Corporation, Irvine, USA). Then the Infinium HumanMethylation450 BeadChip (Illumina Inc., San Diego, USA) was used to measure the methylation level as a beta value ranging from 0 (no methylation) to 1 (complete methylation). All samples passed the quality control. However, we identified 10 samples

as potential gender mismatches which we further excluded in the analysis. The distributions of raw data were already coherent across samples before functional normalization*(18)*, which was done using preprocessFunnorm function from the minfi R package*(29)*. We filtered out cross-reactive probes and sex-chromosome probes. The final number of CpGs that was analysed was 470964. We included a cohort-specific variable in our analysis, for which we adjusted for in order to remove residual cohort-specific effects following the data harmonization and biospecimen randomization. The data was generated at the Epigenetics Group, International Agency for Research on Cancer, Lyon, France.

Covariates

Data on maternal parity, socio-economic status, smoking, pre-pregnancy body mass index, age at birth, newborn gender and gestational age at birth and cohort were self-reported in questionnaires during pregnancy. Based on place of birth of mother and father, approximately 97% of the participants were of European ethnicity.

Cell type correction

We used the Reinius-based Houseman method*(5, 6)* with the *estimateCellCounts* function in the Minfi package*(29)* in R to estimate relative proportions of six white blood cell subtypes (CD4+ T-lymphocytes, CD8+ T-lymphocytes, NK (natural killer) cells, B-lymphocytes, monocytes and granulocytes).

Batch correction

We additionally corrected the analyses for batch effect using surrogate variable analysis (SVA).

Exclusion criteria

In the methylation subset of the EXPOsOMICS cohort we excluded infants born preterm (<37 weeks) (n=28), whose mothers experienced pre-eclampsia (n=2) or diabetes during pregnancy (n=24), from whom we that didn't have complete data for covariates (n=15) and the potential gender mismatches (n=10). The total number of exclusions is 75 because there are 4 overlaps between different exclusion criteria. This information was obtained from questionnaires completed by the mothers shortly after delivery and combined with medical birth records.

Flemish Environment and Health Study (FLEHS1)

Design and study population

The Flemish Environment and Health Study I (FLEHSI) was established for human biomonitoring in different geographically representative samples of the Flemish population in Belgium*(35, 36)*. The FLEHS I birth cohort consists of 1196 mother–child pairs whom were systematically recruited between September 2002 and February 2004 via 25 maternities across Flanders; covering 20% of Flanders' area and 65 different municipalities. Cord blood samples were collected at delivery. Details of the recruitment protocol have been previously reported*(37)*. Inclusion criteria were living for at least five years in the area of interest and being able to fill out Dutch questionnaires. Informed consent was provided by all participating mothers and the campaign was approved by the ethical committee of the University of Antwerp. During follow-up of the cohort at 10 years of age (n=595) data on growth, diet, physical activity, medical conditions and life-style were gathered. A subcohort (n=99) of those followed-up at age 10 years agreed to provide blood samples plus questionnaire data at the age of 11 years.

Birthweight

The outcome of interest was birthweight, which was recorded in grams at birth among other clinical parameters (such as length, Apgar score). We did not have to excluded any of the children based on birthweight being out of range, and there were no missing values.

Methylation measurements

Peripheral blood mononuclear cells (PBMC) were isolated from the blood collected at age 11 years using Lymphoprep™ (Axis-Shield, Oslo, Norway). Whole genomic DNA was isolated from the PBMC fraction. From the 99 blood samples, 92 DNA samples passed the DNA quality check. About 500 ng of gDNA was bisulfite converted using the EZ DNA methylation kit (Zymo Research, Cambridge Bioscience, Cambridge, UK) according to manufacturer's instructions. Genome-wide DNA methylation profiles were generated with Infinium HumanMethylation450 BeadChip Array (Illumina, San Diego, CA, USA) according to the standard Infinium HD Assay Methylation Protocol Guide (Part #15019519, Illumina). The raw methylation intensities for each probe were represented as methylation β-values (ranging from 0, unmethylated, to 1, fully methylated) and extracted from GenomeStudio Methylation Module software without background correction and normalization. Raw data analysis, QC and normalization were performed using "minfi" R-package. In brief, the raw Red/Green channel data from the 450K-Illumina methylation array were read by the 'read.450k.exp' function, converted to methylation values by 'preprocessRaw' and subsequently normalized using 'preprocessSWAN', an implementation of the Subset-quantile Within Array Normalization (SWAN) normalization procedure*(38)*. Principal component analysis and unsupervised clustering were used to check for sample outliers (i.e. 2 because of bad quality). All samples that passed quality controls were loaded into the IMA-package used for further processing. Samples having >75% of CpGs with a detection p-value > 1e-05 were removed (all samples passed this filer). Cg-probes with a detection p-value greater than 0.01 in all samples and cg-probes on the X and Y chromosome were removed. 470,562 sites were retained from the original 485,512 sites.

Covariates
Covariate data (i.e. maternal parity, socio-economic status, smoking, pre-pregnancy body mass index, age at birth, newborn gender and gestational age at birth) were obtained from questionnaires and medical record information for children and their mothers. Maternal smoking status was not included as a covariate in the dichotomous analysis, since none of the mothers of children in the high birthweight group had smoked during pregnancy.

Cell type correction
To estimate the proportion of various cell types in the PBMC samples the statistical deconvolution method described by Houseman and colleagues and implemented in minfi-package as the 'estimateCellCountsMset' function was used*(6)*. Reference methylomes from leukocyte subtypes were obtained from the study of Reinius *et al*.*(5)*

Batch correction
Analyses were additionally adjusted for batch effects by including the batch number (n=2) since there was a gap of a year in between the analyses of 2 batches of samples.

Exclusion criteria
In total 5 participants were excluded: 3 preterm birth (i.e. <37 weeks), 1 maternal diabetes, and 1 pre-eclampsia case. Data on preterm birth, maternal diabetes and pre-eclampsia during pregnancy were obtained from questionnaires completed by the mothers directly after delivery.

GECKO Drenthe
Design and study population
The Groningen Expert Center for Kids with Obesity (GECKO) Drenthe cohort is a population-based prospective birth cohort study in Drenthe, a northern province in the Netherlands. All mothers of infants born between April 2006 and April 2007 were invited to participate during the third trimester of pregnancy. Of all 4,778 infants born in this period, a total of 2,874 newborns (60%) participated in the study. This study has been approved by the Medical Ethical Committee of the University Medical Center Groningen and parents of all participants gave written informed consent. Details about this cohort have been described elsewhere*(39)*.

Birthweight
Newborn birthweight was recorded by obstetricians or midwives in the delivery room or abstracted from obstetric records. We double checked and did not observe birthweight values +/- 5 SD from the mean.

Methylation measurements
Within the GECKO Drenthe birth cohort, we selected 258 infants for the methylation study: 129 exposed to maternal smoking during pregnancy and 129 unexposed to both maternal and paternal smoking during pregnancy*(40)*. From these 258 infants, we used DNA which was extracted from cord blood for the epigenome-wide DNA methylation analyses. To limit batch effects, we randomized all samples over the 96-well plates, based on gender and smoking status. Samples (500 ng per sample) were placed on three 96-well plates. Bisulfite conversion was performed using the EZ-96 DNA methylation kit (Zymo research Corporation, Irvine, USA). Then we used the Infinium HumanMethylation450 BeadChip (Illumina Inc., San Diego, USA) to measure the methylation level as a beta value ranging from zero (no methylation) to one (complete methylation). During the quality control, we excluded two males that clustered in the female group, based on X chromosome betas, which was probably due to maternal blood contamination. We performed Illumina-suggested background normalization, colour correction and Subset-quantile Within Array Normalization (SWAN). We excluded one sample because it did not meet the criteria of ≥99% of the CpGs with detection p value <0.05. This resulted in 129 exposed and 126 unexposed children. We excluded control probes, probes on X or Y chromosomes and probes that did not meet our criteria of a detection p value of <0.05 in ≥99% of the samples, resulting in 465,891 remaining CpGs.

Covariates
Data on maternal parity, socio-economic status, smoking, pre-pregnancy body mass index, age at birth, newborn gender and gestational age at birth were self-reported in questionnaires during pregnancy.

Cell type correction
We used the Reinius-based Houseman method*(5, 6)* with the *estimateCellCounts* function in the Minfi package*(7)* in R*(8)* to estimate relative proportions of six white blood cell subtypes (CD4+ T-lymphocytes, CD8+ T-lymphocytes, NK (natural killer) cells, B-lymphocytes, monocytes and granulocytes).

Batch correction
We additionally corrected the analyses for batch effect by including the Illumina Infinium HumanMethylation450 BeadChip number (n=3).

Exclusion criteria

In the methylation subset of the GECKO Drenthe cohort we excluded infants born preterm (<37 weeks), and whose mothers experienced pre-eclampsia or diabetes during pregnancy, this information was obtained from questionnaires completed by the mothers shortly after delivery and combined with medical birth records.

The Genetics of Glucose regulation in Gestation and Growth (Gen3G)

Design and study population

The Genetics of Glucose regulation in Gestation and Growth (Gen3G) is a population-based prospective birth cohort study in Sherbrooke, Qc, Canada. Between January 2010 and June 2013, we invited pregnant women aged ≥18 years old who visited the blood sampling in pregnancy clinic in Sherbrooke for their first trimester clinical blood samples. 1034 women accepted to participate in our cohort study. This study has been approved by the CHUS Ethics Review Board for Studies with Humans and every participant gave written informed consent before enrolment in the study. Details about this cohort have been described elsewhere*(41)*.

Birthweight

We collected birthweight from clinical obstetric records. We verified and did not observe birthweight values +/- 5 SD from the mean.

Methylation measurements

Within the Gen3G birth cohort we randomly selected 182 infants for the methylation study in the single pregnancies without gestational diabetes. From these 182 infants, we used DNA which was extracted from cord blood for the epigenome-wide DNA methylation analyses. To limit batch effects, we randomly placed all samples (500 ng per sample) over 96-well plates. Bisulfite conversion was performed using the EZ-96 DNA methylation kit (Zymo research Corporation, Irvine, USA). We used the Infinium HumanMethylation450 BeadChip (Illumina Inc., San Diego, USA) to measure methylation levels as a beta value ranging from 0 (no methylation) to 1 (complete methylation). During quality control procedures, we excluded 5 participants that clustered in the wrong sex group and one that was considered an outlier from the MDS plot. We excluded 6 samples because they did not meet the criteria of ≥99% of the CpGs with detection p value <0.01. Finally, we removed 8 samples from this analysis, because of missing values (n=6) or because they were not born at term (n=2). Thus, we conducted this analysis in 162 children. We performed DASEN normalization from the watermelon package. We excluded probes that did not meet our criteria of a detection p value of <0.01 in ≥80% of the samples, resulting in 482,992 remaining CpGs.

Covariates

Data on maternal parity, smoking, age at birth were self-reported in questionnaires during first trimester of pregnancy. Maternal pre-pregnancy BMI was calculated with self-reported pre-gestational weight and height measured at first trimester. Newborn gender and gestational age at delivery were abstracted from obstetric records. Maternal social class was not available in our study.

Cell type correction

We used the Reinius-based Houseman method*(5, 6)* with the *estimateCellCounts* function in the Minfi package*(7)* in R*(8)* to estimate relative proportions of six white blood cell subtypes (CD4+ T-lymphocytes, CD8+ T-lymphocytes, NK (natural killer) cells, B-lymphocytes, monocytes and granulocytes).

Batch correction

We additionally corrected the analyses for batch effect by including the Illumina Infinium HumanMethylation450 Sample plate number in our regression analyses.

Exclusion criteria

For the main analysis we excluded n=2 infants born preterm (<37 weeks). This information was obtained from medical birth records. For our study using Illumina Infinium HumanMethylation450 we had a priori decided to select samples from babies born from mothers who did not develop gestational diabetes (based on oral glucose tolerance test) and without pre-eclampsia (medical records).

The Generation R Study

Design and study population

The Generation R Study is a prospective population-based cohort in Rotterdam, the Netherlands*(42)*. All pregnant women residing in Rotterdam with a delivery date between April 2002 and January 2006 were invited to participate. The Medical Ethical Committee of Erasmus MC, University Medical Center Rotterdam, approved the study and an informed consent was obtained for all participating children. In total, 9,778 mothers were enrolled in the study*(42)*.

Birthweight

Birthweight was obtained from community midwife and hospital registries. All birthweight values were within +/-5 SD from the mean. Birthweight was analysed in grams.

Methylation measurements

DNA extracted (using the salting-out method) from cord blood and blood samples from children aged around 10 years of European-ancestry was used for this analysis. 500 ng DNA per sample underwent bisulfite conversion using the EZ-96 DNA Methylation kit (Shallow) (Zymo Research Corporation, Irvine, USA). Samples were plated onto 96-well plates in no specific order. Samples were processed with the Illumina Infinium HumanMethylation450 BeadChip (Illumina Inc., San Diego, USA), which analyses methylation at 485,577 CpGs. Quality control of analyzed samples was performed using standardized criteria, separately for the cord blood samples and the child samples, as the latter were added at a later stage.

Quality control and normalisation of cord blood samples

For the cord blood samples, reasons for sample exclusions were sample call rate <99%, colour balance >3, low staining efficiency, poor extension efficiency, poor hybridization performance, low stripping efficiency after extension, poor bisulfite conversion and gender mismatch. After quality control, 969 samples remained in the analysis. Probes with a single nucleotide polymorphism in the single base extension site with a frequency of >1% in the GoNLv4 reference panel were excluded*(43)*, as were probes with non-optimal binding (non-mapping or mapping multiple times to either the normal or the bisulphite-converted Genome*(44)*, resulting in the exclusion of 49,564 probes, leaving a total of 436,013 probes in the analysis. We ran DASES normalization using a pipeline adapted from that developed by Touleimat and Tost*(26)*. DASES normalization includes background adjustment, between-array normalization applied to type I and type II probes separately, and dye bias correction applied to type I and type II probes separately and is based on the DASEN method described by Pidsley *et al*. but adds the dye bias correction, which is not included in DASEN*(45)*.

Quality control and normalisation of child blood samples

Preparation and normalization of the HumanMethylation450 BeadChip array data was performed according to the CPACOR workflow[1] using the software package R[2]. In detail, the idat files were read using the minfi package. Probes that had a detection p-value above background (based on sum of methylated and unmethylated intensity values) ≥ 1E-16 were set to missing per array. Next, the intensity values were stratified by autosomal and non-autosomal probes and quantile normalized for each of the six probe type categories separately: type II red/green, type I methylated red/green and type I unmethylated red/green. Beta values were calculated as proportion of methylated intensity value on the sum of methylated+unmethylated+100 intensities. Arrays with observed technical problems such as failed bisulfite conversion, hybridization or extension, as well as arrays with a mismatch between sex of the proband and sex determined by the chr X and Y probe intensities were removed from subsequent analyses. Additionally, only arrays with a call rate > 95% per sample were processed further. The final dataset contained 425 samples at age 10.

Covariates

Data on maternal age, educational level and parity were collected by questionnaires in early pregnancy. Maternal body mass index was assessed at intake. Maternal smoking during pregnancy was assessed by questionnaires in each trimester of pregnancy. Gestational age and sex of the child were determined by ultrasound during pregnancy as described previously*(46)*. Current age and BMI of the children included in the sensitivity analysis were recorded/measured at our research center.

Cell type correction

Cell type correction was applied using the reference-based Houseman method*(6)* in the minfi Package*(29)* in R*(8)*. This method estimates the relative proportions of six white blood cell subtypes (CD4+ T-lymphocytes, CD8+ T-lymphocytes, NK (natural killer) cells, B-lymphocytes, monocytes and granulocytes), based on a standard reference population*(5)*.

Batch correction

We additionally corrected the analyses for batch effect by adjusting for plate number.

Exclusion criteria

For the main analysis we excluded n=165 participants: 23 infants born preterm (<37 weeks) and 49 postterm (>42 weeks), and whose mothers experienced pre-eclampsia (n=81) or diabetes (n=12) during pregnancy. This information was obtained from questionnaires completed by the mothers shortly after delivery and combined with medical birth records.

Glycyrrhizin in Licorice (Glaku)

Design and study population

The study participants were included from an urban community-based birth cohort Glycyrrhizin in Licorice (GLAKU)*(47)*. It is a prospective study cohort of 1049 infants born between March and November of 1998 at the Helsinki University Hospital and Helsinki City Maternity Hospital, Finland. A primary objective of the cohort was to examine the effects of maternal licorice consumption during pregnancy on their offspring's developmental outcomes. Between 2009 and 2011, all 920 cohort members who had given permission to be contacted and whose addresses were traceable were invited to participate in a follow-up study; 692 (75.2%) were contacted by phone, and 451 (65.2% of the women who were contacted) participated. Of the offspring of the participants to the follow-up, 240 children donated blood and there were 216 children (54.2% girls) in the final analytical sample. Their mean age was 12.4 years (SD=0.5, range 11.1-13.1 years). Details of the initial sample collection and follow-up in 2009-2011 have been reported elsewhere*(47, 48)*. The study protocol was approved by the ethical committees of the City of Helsinki and the Uusimaa Hospital District. Written informed consent was obtained from the mothers after delivery and from the parent/guardian and adolescent at the follow-up assessment.

Birthweight
Infant weight (kg) was measured immediately after birth with a standard electronic scale by midwives in the delivery room or abstracted from obstetric records. There were no birthweight values +/- 5 SD from the mean.

Methylation measurements
Blood was drawn in 2009-2011 visit in participating children and DNA was extracted from for the epigenome-wide DNA methylation analyses. Bisulfite conversion was performed using the EZ-96 DNA methylation kit (Zymo research Corporation, Irvine, USA). 240 blood samples were ran on Illumina EPIC Methylation arrays (Illumina Inc., San Diego, USA). The quality control pipeline was set up using the R-package minfi. Methylation beta-values were normalized using the funnorm function. One IDs showed density artefacts after normalization and was removed from further analysis. We excluded any probes on chromosome X or Y, probes containing SNPs and cross-hybridizing probes according to Chen *et al.(13)*, Price *et al.(49)* and McCartney *et al.(50)* Furthermore, any CgGs with a detection p-value > 0.01 in at least 25% of the samples were excluded. The final dataset contains 812,943 CpGs and 239 IDs.

Covariates
Data on newborn gender was extracted from the social security number and gestational age at birth was estimated based on ultrasound. Data on smoking, pre-pregnancy body mass index, parity, and age at birth were self-reported in questionnaires during pregnancy. In addition, data on maternal education, child BMI, and age at the follow-up were self-reported. We also used first three MDS components based on genome-wide genotyping with Illumina OmniExpressExome 1.2 array data to adjust for population stratification.

Cell type correction
We used the Reinius-based Houseman method*(5, 6)* with the *estimateCellCounts* function in the Minfi package*(7)* in R*(8)* to estimate relative proportions of six white blood cell subtypes (CD4+ T-lymphocytes, CD8+ T-lymphocytes, NK (natural killer) cells, B-lymphocytes, monocytes and granulocytes).

Batch correction
We used ComBat to check and adjust for the batch effects (row).

Exclusion criteria
For the main analysis we excluded n=7 due to preterm birth (<37 weeks based on ultrasound estimate of gestational age), which is 2.9% of the Glaku participants with methylation and birthweight data. This information was obtained from medical birth records.

Genome-Wide Population-based Association Study of Extremely Overweight Young Adults (GOYA)
Design and study population
The Genome-Wide Population-based Association Study of Extremely Overweight Young Adults (GOYA) study is described previously by Paternoster *et al.(51, 52)* It is based on the Danish National Birth Cohort (DNBC) that included 92,000 pregnant women and their pregnancies during 1996-2002. Of 67,853 women who had given birth to a live born infant, had provided a blood sample during pregnancy and had BMI information available, 3.6% of these women with the largest residuals from the regression of BMI on age and parity (all entered as continuous variables) were selected for GOYA. The BMI for these 2451 women ranged from 32.6 to 64.4. From the remaining cohort a random sample of similar size (2450) was also selected. DNA methylation data were generated for the offspring of 1000 mothers in the GOYA study. I.e. “cases” had mothers with a BMI>32 and “controls” were sampled from the normal BMI distribution (can include mothers with a BMI>32). All participants in the DNBC gave written informed consent and the collection and use of their data has ethics approval. Ethnicity was obtained using register data.

Birthweight
Information on birthweight was recorded by healthcare professionals at the time of birth and extracted from birth records within the National Hospital Discharge Registry. Within GOYA, there were no values +/- 5SD from the mean.

Methylation measurements
Cord blood was collected according to standard procedures, spun and frozen at -80˚C. DNA methylation analysis and data pre-processing were performed at the University of Bristol. Following extraction, DNA was bisulfite converted using the Zymo EZ DNA MethylationTM kit (Zymo, Irvine, CA). Following conversion, the genome-wide methylation status of over 485,000 CpGs was measured using the Illumina Infinium® HumanMethylation450k BeadChip assay according to the standard protocol. The arrays were scanned using an Illumina iScan and initial quality review was assessed using GenomeStudio (version 2011.1). The level of methylation is expressed as a “Beta” value (β-value), ranging from 0 (no cytosine methylation) to 1 (complete cytosine methylation). Samples from all time-points in ARIES were distributed across slides using a semi-random approach (sampling criteria were in place to ensure that all time-points were represented on each array) to minimize the possibility of confounding by batch effects. Samples failing quality control (average probe detection p-value ≥ 0.01) were repeated. As an additional quality control step genotype probes on the HumanMethylation450k were compared between samples from the same individual and against SNP-chip data to identify and remove any sample mismatches. Data were normalized using the functional normalization approach in the Minfi R package.

We removed probes that had a detection P-value >0.05 for >5% of samples, probes on the X or Y chromosomes and SNPs (rs probes). 473864 probes remained.

Covariates
Data on maternal parity, socio-economic status, smoking and pre-pregnancy body mass index were collected via a telephone interview at around 16 weeks' gestation. Maternal age was derived from the mother's report of her own date of birth. Newborn sex and gestational age at birth were extracted from birth records.

Cell type correction
We used the Reinius-based Houseman method*(5, 6)* with the *estimateCellCounts* function in the Minfi package*(7)* in R*(8)* to estimate relative proportions of six white blood cell subtypes (CD4+ T-lymphocytes, CD8+ T-lymphocytes, NK (natural killer) cells, B-lymphocytes, monocytes and granulocytes).

Batch correction
Ten surrogate variables were generated using the sva package in R and included in models to adjust for technical batch.

Exclusion criteria
For the main analysis we excluded n=25 participants: 12 infants born preterm (<37 weeks), and infants whose mothers experienced pre-eclampsia (n=10) or diabetes (n=5) during pregnancy, there was some overlap between these. This information was obtained from questionnaires completed by the mothers shortly after delivery and combined with medical birth records.

Healthy Start
Design and study population
The Healthy Start study is an ongoing, prospective pre-birth cohort in Colorado, USA*(53, 54)*. Eligible pregnant women were recruited from the University of Colorado Hospital Outpatient Pavilion, 2009-2014. Eligibility criteria were: age 16 years or older, singleton pregnancy, no prior stillbirths, and gestational age < 24 weeks. We recruited 1,410 pregnant women, approximately 50% of those eligible. Study procedures were approved by the Colorado Multiple Institutional Review Board and written informed consent was obtained from all participating mothers.

Birthweight
Birthweight was obtained from the medical record at delivery.

Methylation measurements
Umbilical cord blood was collected at delivery. Eligibility for DNA methylation analysis was determined by the availability of cord blood and maternal blood and urine samples during pregnancy. Methylation analysis of cord blood samples (N=600) was conducted using the Illumina Infinium HumanMethylation450 BeadChip, and processed in the University of Colorado Genomics Core lab. Samples with predicted child sex inconsistent with reported sex were excluded. Probes with high detection p-value (>0.05) or low beadcount (<3 in at least 5% of the samples) were excluded. All analyses were conducted in R version 3.3.0*(8)*. The preprocessQuantile function in the R package Minfi*(29)* was used for normalization. The total number of CpGs analysed was 484,261.

Covariates
Models were stratified by maternal race/ethnicity group (non-Hispanic white, Hispanic, or non-Hispanic African American). Models were adjusted for the following covariates: gestational age at delivery and infant sex (obtained from the medical record at delivery), maternal age, parity, pre-pregnancy BMI, education completed, smoking during pregnancy (obtained from the mother via questionnaire or from the prenatal medical record).

Cell type correction
We used the estimateCellCounts function in Minfi to estimate relative proportions of six white blood cell subtypes (CD4+ T-lymphocytes, CD8+ T-lymphocytes, NK (natural killer) cells, B-lymphocytes, monocytes and granulocytes).

Batch correction
We corrected for batch effects using ComBat*(16)*.

Exclusion criteria
For the main analysis we excluded n=19 infants born preterm (<37 weeks), and/or whose mothers experienced pre-eclampsia (n=21) or diabetes (n=33) during pregnancy, and 80 participants missing information on maternal smoking during pregnancy. We additionally excluded n=28 participants who belonged to racial/ethnic groups other than the 3 major groups, due to small numbers. This information was obtained from questionnaires completed by the mothers shortly during pregnancy or shortly after delivery, and combined with information from medical birth records. Finally, we excluded 4 participants for whom reported child sex was inconsistent with predicted child sex based on DNA methylation. The total sample size after exclusions was 432 (240 non-Hispanic white, 115 Hispanic, 77 non-Hispanic African American).

Infancia y Medio Ambiente (INMA)

Design and study population

The INMA—INfancia y Medio Ambiente—(Environment and Childhood) Project is a network of birth cohorts in Spain that aims to study the role of environmental pollutants in air, water and diet during pregnancy and early childhood in relation to child growth and development*(55)*. Mothers were enrolled at week 12 of pregnancy from 1997 to 2008 in seven regions of Spain (Flix, Granada, Menorca, Asturias, Gipuzkoa, Sabadell and Valencia). The cohort consisted of 3,768 children at birth. During the follow-up visits information on environmental exposures and health outcomes (reproductive, growth and obesity, lung function, allergies and neurovelopment) were assessed through questionnaires, biomarker measurements, clinical data, and physical exploration. The study website contains details of the design and data available in INMA project (http://www.proyectoinma.org/). The study was approved by the Ethical Committees of each participating centre and written consent was obtained from parents.

In this study, DNA methylation data was available for 166 cord blood samples from INMA Sabadell subcohort.

Birthweight

Birthweight was recorded by specially trained midwives at delivery. We did not observe birthweight values +/- 5 SD from the mean for the gestational age or sex. We only participated in the main analysis using birthweight as continuous. The dichotomous analyses were excluded of this paper as only seven of our children were categorized as high (>4000g) birthweight.

Methylation measurements

Cord blood was extracted using the Chemagen kit (Perkin Elmer). DNA concentration was determined by a NanoDrop spectrophotometer (Thermo Scientific) and with the Quant-iT PicoGreen dsDNA Assay Kit (Life Technologies).

Blood methylation data was produced in the Genome Analysis Facility of the University Medical Center Groningen (UMCG) in Holland as part of the MeDALL (Mechanisms of the Development of Allergy) project. Samples were randomized and processed following the Illumina protocol for the Infinium HumanMethylation450 BeadChip. Briefly, 500 ng of DNA was bisulfite-converted using the EZ 96-DNA methylation kit, and DNA methylation was measured through hybridization on the BeadChips.

DNA methylation data were pre-processed in R*(8)* using minfi*(29)*, from the original idat files extracted from the HiScanSQ scanner. Samples that did not provide significant methylation signals in more than 10% of probes (detection P>0.01) were excluded from further analysis. Samples were also excluded in cases of low staining efficiency, low single base extension efficiency, low stripping efficiency of DNA from probes after single base extension, poor hybridization performance, poor bisulphite conversion and high negative control probe staining. Further, we assessed the methylation distribution of the X-chromosome to verify gender. Finally, we normalized the beta values using dasen*(45)*. We removed 65 SNPs probes, the probes on sex chromosomes, potential cross reactive probes, and probes containing SNPs at the target CpGs with a MAF>10% (polymorphic) were excluded*(13)*. A total of 166 subjects and 439,306 CpG probes were analyzed.

Covariates

We calculated gestational age from the date of the last menstrual period (LMP) reported at recruitment and confirmed using estimates based on the first ultrasound examination (about 12th week of gestation). When the difference between the LMP reported at recruitment and estimated from the ultrasound was ≥ 7 days, we estimated LMP using the crown-rump length*(56)*. Data on age at birth, and maternal height and pre-pregnancy weight (for BMI calculations) were collected from a self-reported using a questionnaire at enrolment (week 12 of pregnancy). Maternal socioeconomic status was based on maternal occupation at pregnancy, it was categorized into three levels: low (levels V/VI semi-skilled/unskilled occupations), medium (levels III/IV skilled manual/non-manual) or high (managers/technicians). Pregnant women were asked whether they were current smokers (at week 32 of pregnancy) and if so, how much. They were also asked if they had stopped smoking due to pregnancy and when (before pregnancy or at what month of pregnancy). Any smoking was defined as smoking any number of cigarettes at any time during pregnancy.

Cell type correction

We used the *estimateCellCounts* function from minfi which estimates a constrained projection using quadratic programming*(6)* we estimated six white blood cell subtypes (CD4+ T-lymphocytes, CD8+ T-lymphocytes, NK (natural killer) cells, B-lymphocytes, monocytes and granulocytes) using the Reinius reference*(5)*.

Batch correction

Batch correction was attained including the significant (permutation p-value< 10^{-4}) principal components derived from the 613 negative control probes presented in 450K arrays. After 10.000 permutations 5 PCs were retained. The beta-values were batch corrected incorporating these 5 PCs and calculating the residuals of the linear model.

Exclusion criteria

For the main analysis we excluded n=17 participants: 5 infants born preterm (<37 weeks), and whose mothers experienced pre-eclampsia (n=6) or diabetes (n=15) during pregnancy. This information was obtained from questionnaires completed by the mothers shortly after delivery and combined with medical birth records.

Gene expression analysis

INMA gene expression data was collected at 4 years of age. Whole blood was collected in PAXGene tubes and extracted using the kit recommended by the company. All samples had a RNA Integrity Number (RIN) higher than seven. Gene expression data was obtained using Affymetrix Human Transcriptome Array 2.0 at the European Institute for Systems Biology and Medicine in Lyon as part of the MeDALL project. Gene expression was normalized with the RMA algorithm using the ExpressionConsole Software from Affymetrix and probes were clustered to the transcript level. Expression transcripts were annotated using version 36 of Affymetrix annotation. Four samples were excluded because they were outliers defined as more than 3 SD from the mean for PC1 or PC2 (N=4). The final sample size was 112.

DNA methylation and DNA extraction has been described elsewhere in the text. In brief, the beta-values that passed the quality control filtering were background corrected using methylumi-noob and functionally normalized*(18)*. As different laboratory batches were processed for this specific analysis, we applied ComBat*(16)* to reduce this undesired technical variability. Next, the effect of sex and blood cell proportions estimates were controlled out in a linear regression model. Cell proportions were estimated using the constrained projection quadratic programming (CP/QP) approach of Houseman*(6)*, using the estimateCellCounts function in the minfi R package*(8, 29)*. We used the Reinius panel as the reference*(5)*.

Only transcripts inside a 500 kb window of a selected CpG (250 kb downstream and 250 kb upstream) were considered in the analysis. The model was controlled for technical and unwanted biological variation when estimating gene expression residuals, 13 surrogate variables were detected and added to the model including sex and 6 cell estimates. Surrogate variables were estimated using the sva R package*(57)*. Cell estimates used the Reinius dataset as the reference*(5)*.

Isle of Wight Birth Cohort (IOW F1)

Design and study population

A whole population birth cohort was established on the Isle of Wight, UK, in 1989 to prospectively study the natural history of allergic diseases from birth onwards*(58)*. Both the Isle of Wight and the study population are 99% Caucasian. Ethics approvals were obtained from the Isle of Wight Local

Research Ethics Committee (now named the National Research Ethics Service, NRES Committee

South Central –Southampton B) at recruitment and for the 1, 2, 4, 10 and 18 years follow-up. Of the

1536 children born between January 1, 1989, and February 28, 1990, written informed consent was obtained from parents to enroll 1456 newborns. Children were followed up at the ages of 1 (n =

1167), 2 (n = 1174), 4 (n = 1218), 10 (n = 1373), and 18 years (n = 1313). Demographic information of parents and offspring, status of allergic diseases, phenotypic measures on allergic sensitization, IgE, and lung function, and environmental exposures, along with other phenotypic measures, were collected at birth and updated at each follow-up.

Birthweight

Newborn birthweight was collected from stored clinical records. The outliers for observe birthweight values +/- 5 SD from the mean was checked. For the main analysis model birthweights were treated as continuous variables in grams and for the dichotomous model birthweights were categorised in high (>4000g) versus normal (2500-4000g) birthweight.

Methylation measurements

For 367 aged 18 years subjects, we measured DNA methylation from whole blood processed with the Illumina Infinium HumanMethylation450 BeadChip (Illumina Inc., San Diego, USA). CPACOR*(59)* pipeline was used for QC and normalisation of the data. Methylation markers on 65 single nucleotide polymorphism (SNP) and sex chromosomes were removed. We applied Illumina background

Correction to all intensity values. Any intensity values having detection p-values >= 10-16 were set as missing data. Samples with call rate < 98% were excluded. After the QC, 461,230 sites remain for the subsequent analysis. A quantile normalisation was applied using limma on intensity values

separately based on six different probe-type categories (Type-I M red, Type-I U red, Type-I M green,

Type-I U green, Type-II red, and Type-II green). Beta values were then calculated from these normalised intensity values

Covariates

Covariates were collected via questionnaires collected at recruitment, before and during pregnancy.

Maternal age was derived from mothers' date of birth. Child's gender and gestational age at birth were collected from stored clinical information. In case of maternal BMI, we used BMI at 1st trimester. Maternal smoking status and parity were collected

from the responses from the questionnaires. Maternal smoking status in pregnancy (Yes/No) was defined as any smoking in pregnancy or no smoking in pregnancy. Socioeconomic status was defined using maternal socioeconomic cluster information (high, low, low-low, low-mid, and mid).

Cell type correction
We used the Reinius-based Houseman method*(5, 6)* with the estimateCellCounts function in the Minfi package*(7)* to estimate relative proportions of six white blood cell subtypes (CD4+ T-lymphocytes, CD8+ T-lymphocytes, NK (natural killer) cells, B-lymphocytes, monocytes and granulocytes).

Batch correction
Indicator of different batches that DNA methylation data were generated and ComBat*(16)* was used to adjust for batch effect.

Exclusion criteria
In the methylation subset of the samples were excluded infants born preterm (<37 weeks), and whose mothers experienced pre-eclampsia or diabetes during pregnancy. This information was obtained from questionnaires completed by the mothers shortly after delivery and combined with medical birth records.

Isle of Wight Third Generation (IOW F2)
Design and study population
The recruitment of newborns started from April 2010. Data used in the analyses were from infants born between April 2010 to May 2014*(58)*. In total, 200 newborns were recruited such that at least one of their parents is in the IOW birth cohort (IOW F1) and the recruitment is ongoing. For each infant, along with other phenotypic information such as gender and birthweight, status of wheezing and eczema was recorded, measures of wheal size from skin prick test as well as IgE were recorded.

Birthweight
Newborn birthweight was collected from stored clinical records. The outliers for observe birthweight values +/- 5 SD from the mean was checked. For the main analysis model birthweights were treated as continuous variables in grams and for the dichotomous model birthweights were categorised in high (>4000g) versus normal (2500-4000g) birthweight.

Methylation measurements
For 118 subjects, we measured DNA methylation from whole blood processed with the Illumina Infinium HumanMethylation450 BeadChip (Illumina Inc., San Diego, USA). CPACOR*(59)* pipeline was used for QC and normalisation of the data. Methylation markers on 65 single nucleotide polymorphism (SNP) and sex chromosomes were removed. We applied Illumina background

Correction to all intensity values. Any intensity values having detection p-values >= 10-16 were set as missing data. Samples with call rate < 98% were excluded. After the QC, 389,828sites remain for the subsequent analysis. A quantile normalisation was applied using limma on intensity values

separately based on six different probe-type categories (Type-I M red, Type-I U red, Type-I M green,

Type-I U green, Type-II red, and Type-II green). Beta values were then calculated from these normalised intensity values

Covariates
Covariates were collected via questionnaires collected at recruitment, before and during pregnancy.

Maternal age was derived from mothers' date of birth. Child's gender and gestational age at birth were collected from stored clinical information. In case of maternal BMI, we used BMI at 1st trimester. Maternal smoking status and parity were collected from the responses from the questionnaires. Maternal smoking status in pregnancy (Yes/No) was defined as any smoking in pregnancy or no smoking in pregnancy. Socioeconomic status was defined using maternal education: 1) left before general certificate of secondary education, 2) completed education at 16 years, 3) completed education at 18 years, and 4) Other, e.g., vocational training. Subjects who completed education at 16 years or above were included into group 1.

Cell type correction
We used the Reinius-based Houseman method*(5, 6)* with the estimateCellCounts function in the Minfi package*(7)* to estimate relative proportions of six white blood cell subtypes (CD4+ T-lymphocytes, CD8+ T-lymphocytes, NK (natural killer) cells, B-lymphocytes, monocytes and granulocytes).

Batch correction
Indicator of different batches that DNA methylation data were generated and ComBat*(16)* was used to adjust for batch effect.

Exclusion criteria
In the methylation subset of the samples were excluded infants born preterm (<37 weeks), and whose mothers experienced pre-eclampsia or diabetes during pregnancy. This information was obtained from questionnaires completed by the mothers shortly after delivery and combined with medical birth records.

LifeLines Cohort study

Design and study population

The LifeLines Cohort Study is a multi-disciplinary prospective large population-based cohort study examining in a unique three-generation design the health and health-related behaviors of 167,729 persons living in the North of the Netherlands. It employs a broad range of investigative procedures in assessing the biomedical, socio-demographic, behavioral, physical and psychological factors which contribute to the health and disease of the general population, with a special focus on multi-morbidity and complex genetics. In addition, a biobank was established as a resource for research on complex interactions between environmental, phenotypic and genomic factors in the development of chronic diseases and healthy ageing*(60, 61)*. Between 2006 and 2013, inhabitants of the northern part of The Netherlands and their families were invited to participate, thereby contributing to a three-generation design. Participants visited one of the LifeLines research sites for a physical examination and completed extensive questionnaires. Baseline data were collected for 167,729 participants, aged from 6 months to 93 years. Follow-up visits are scheduled every 5 years, and in between participants receive follow-up questionnaires. This study has been approved by the Medical Ethical Committee of the University Medical Center Groningen and all participants gave written informed consent.

Birthweight

Birthweight was measured by questionnaire. All subjects with a self-reported birthweight between 950 and 5000 grams were included. For the main analysis model we treated birthweight as a continuous trait in grams and for the dichotomous model we categorised birthweight in high (>4000g) versus normal (2500-4000g) birthweight. We had complete data (including all covariates and methylation data) on 745 and 728 subjects for the continuous and dichotomous analyses respectively.

Methylation measurements

The methylation levels at the baseline assessment of LifeLines were determined for use in a study on environmental exposures, DNA methylation, and respiratory health. In total, 1,656 subjects were selected based on having complete data on sex, age, height, smoking history (pack-years, never- or current-smoker), airway obstruction (FEV1/FVC<70%), and occupational related exposures. The Illumina Infinium Human Methylation 450K arrays were used to define genome-wide DNA methylation levels in whole blood at >450.000 specific CpGs. We randomized 1,656 subjects based on sex, exposure, and airway obstruction over the chips. Using 500 ng DNA for each sample, we first performed a bisulphite conversion using the EZ- 96 DNA methylation kit (Zymo research Corporation, Irvine, USA), which was validated using commercially available bisulphite conversion 1 control samples (Zymo Research Corporation, Irvine, USA). After this step the samples were processed according to the Illumina 450K protocol (Illumina Inc., San Diago, USA). Quality-control (QC) steps were performed using the Minfi package in R*(29)*, and included the removal of samples with >1% of all probes having a detection p-value >0.01, and samples with an incorrect sex or SNP prediction. We removed single probes with a detection p-value >0.01, sex chromosome probes, cross-reactive probes, probes measuring SNPs, and probes where the CpG itself or the single base extension (SBE) site is a SNP*(13)*. The data were normalized using DASEN implemented in the wateRmelon package in R*(45)*. The final data set contained data for 1,622 subjects and 420,938 CpG probes. Beta-values were used to represent DNA methylation levels, which is the ratio between the intensities of methylated versus unmethylated probes, ranging from 0 to 1.

Covariates

Data on age (at the time of blood collection), gender, gestational age, maternal smoking, maternal age at delivery, parity, current social class (defined by educational level), and current smoking were determined by questionnaires. Current BMI was measured during the baseline visit. Data on maternal social class and pre-pregnancy BMI were not available. All subjects were Dutch Caucasians, so the analysis was not adjusted for etnicity.

Cell type correction

The minfi package was used to calculate the cell proportions using the Houseman method*(6)*. These include: CD8T, CD4T, NK, Bcell, Mono and Gran.

Batch correction

To estimate possible batch effects, a principal component (PC) analysis was performed using the control probes included on the 450K chip*(59)*. We included seven PCs in the final model that explained >1% of the variance, and captured in total >95% of the technical variance.

Exclusion criteria

In the methylation subset of the LifeLines cohort we excluded infants born preterm (<37 weeks).

Norwegian Mother and Child Cohort Study (MoBa1, MoBa2 and MoBa3)

Design and study population

Participants represent three subsets of mother-offspring pairs from the national Norwegian Mother and Child Cohort Study (MoBa)*(62)*. MoBa is a prospective population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health. The years of birth for MoBa participants ranged from 1999-2009. MoBa mothers provided written informed consent. Each

subset is referred to here as MoBa1, MoBa2, and MoBa3. MoBa1 is a subset of a larger study within MoBa that included a cohort random sample and cases of asthma at age three years*(63)*. We previously reported an association between maternal smoking during pregnancy and differential DNA methylation in MoBa1 newborns*(64)*. We subsequently measured DNA methylation in additional newborns (MoBa2) in the same laboratory (Illumina, San Diego, CA)*(65)*. MoBa2 included cohort random samples plus cases of asthma at age seven years and non-asthmatic controls. MoBa3 was designed to evaluate the association between differential cord blood DNA methylation and later childhood cancer status. Methylation measurements for MoBa3 were made at IARC. Years of birth were 2002-2004 for children in MoBa1, 2000-2005 for MoBa2, and 200-2008 for MoBa3. The establishment and data collection in MoBa has obtained a license from the Norwegian Data Inspectorate and approval from The Regional Committee for Medical Research Ethics. All three studies were approved by the Regional Committee for Ethics in Medical Research, Norway. In addition, MoBa1 and MoBa2 were approved by the Institutional Review Board of the National Institute of Environmental Health Sciences, USA.

Birthweight

In MoBa, birthweight was collected in the Medical Birth Registry of Norway (MBRN). The distribution was checked for values outside +/-5SD for removal (0 in MoBa1, MoBa2 and MoBa3). Both the continuous and the dichotomous high (>4000g) versus normal (2500-4000g) birthweight models were analyzed in MoBa1, MoBa2, and MoBa3.

Methylation measurements

Details of the DNA methylation measurements and quality control for the MoBa1 participants were previously described*(66)* and the same protocol was implemented for the MoBa2 participants. Briefly, umbilical cord blood samples were collected and frozen at birth at -80°C. All biological material was obtained from the Biobank of the MoBa study*(66)*. Bisulfite conversion was performed using the EZ-96 DNA Methylation kit (Zymo Research Corporation, Irvine, CA) and DNA methylation was measured at 485,577 CpGs in cord blood using Illumina's Infinium HumanMethylation450 BeadChip*(67)*. Raw intensity (.idat) files were handled in R using the minfi package to calculate the methylation level at each CpG as the beta-value (β=intensity of the methylated allele (M)/(intensity of the unmethylated allele (U) + intensity of the methylated allele (M) + 100)) and the data was exported for quality control and processing. Probe and sample-specific quality control was performed in the MoBa1, MoBa2, and MoBa3 datasets separately. Similar protocols were applied to MoBa1 and Moba2, as follows: Control probes (N=65) and probes on X (N=11 230) and Y (N=416) chromosomes were excluded in both datasets. Remaining CpGs missing > 10% of methylation data were also removed (N=20 in MoBa1, none in MoBa2). Samples indicated by Illumina to have failed or have an average detection p value across all probes < 0.05 (N=49 MoBa1, N=35 MoBa2) and samples with gender mismatch (N=13 MoBa1, N=8 MoBa2) were also removed. For MoBa1 and MoBa2, we accounted for the two different probe designs by applying the intra-array normalization strategy Beta Mixture Quantile dilation (BMIQ)*(19)*. After quality control exclusions, the sample sizes were 1,068 for MoBa1 and 685 for MoBa2. For MoBa3, samples were completely randomized, and bisulfite conversion and methylation measurements were performed by the Epigenetics Group at IARC (Lyon, France). Similar data quality control and processing was applied with some slight differences. Methylation features were filtered from (i) cross-reactive probes, (ii) probes mapping to sex chromosomes and (iii) probes overlapping with a known single nucleotide polymorphism (SNP) with an allele frequency of at least 5% in the overall population (all ethnic groups), resulting in the exclusion of 36 231 probes. Data quality was further assessed using box plots for the distribution of methylated and unmethylated signals, and multidimensional scaling plots and unsupervised clustering were used to check for sample outliers. After background correction and color-bias adjustment, type I and type II probe distributions were aligned using the intra-sample BMIQ normalization*(19)* from the watermelon package. After quality control, the sample size for MoBa3 was 253.

Covariates

For all three datasets, information on gestational age, child's sex, maternal age, smoking during pregnancy, education, pre-pregnancy BMI, and parity was collected via questionnaires completed by the mother or from birth registry records as previously described (4). Gestational age, maternal age, and pre-pregnancy BMI were included as continuous variables. Child's sex and parity were included as dichotomous variables. Maternal smoking status during pregnancy was classified into three groups: non-smoker, stopped smoking in early pregnancy, and smoked throughout pregnancy. Maternal educational level was categorized into four groups based on years of education: less than high school/secondary school, high school/secondary school completion, some college or university, or 4 years of college/university or more.

The current analyses include the children who had cord blood DNA methylation measurements that passed quality control, birthweight and covariate data (N=1025 from MoBa1; N=642 from MoBa2; N=205 from MoBa3). Each dataset was analysed independently.

Cell type correction

We used the Houseman method*(6)* with the estimateCellCounts function in the Minfi package*(7)* in R*(8)* to estimate relative proportions of six white blood cell subtypes (CD4+ T-lymphocytes, CD8+ T-lymphocytes, NK (natural killer) cells, B-lymphocytes, monocytes and granulocytes).

Batch correction

The Empirical Bayes method via ComBat was applied separately in MoBa1, MoBa2 and MoBa3 for batch correction using the sva package in R*(57)*.

Exclusion criteria

For the main analysis we excluded infants born preterm (<37 weeks; N=37 for MoBa1, N=30 for MoBa2, and N=0 for MoBa3), and whose mothers experienced pre-eclampsia (N=34 for MoBa1, N=26 for MoBa2, and N=0 for MoBa3) or diabetes (N=18 for MoBa1, N=2 for MoBa2, and N=0 for MoBa3) during pregnancy. This information was collected via questionnaires completed by the mother or from birth registry records as previously described*(62, 68)*.

Norway Facial Clefts Study (NCL)

Design and study population

The Norway Facial Clefts Study is a national population-based case-control study of cleft lip and cleft palate, disorders characterized by the incomplete fusion of the lip and/or palate during development. The study design has been previously described in detail*(69)*. Study approval was obtained by the Norwegian Data Inspectorate and Regional Medical Ethics Committee of Western Norway and informed consent was provided by both the mother and father. Briefly, between the years of 1996 and 2001 all families of newborns referred for cleft surgery in Norway were contacted and, of those eligible, 88% agreed to participate (N=573). Controls were selected by a random sampling of roughly 4 per 1000 live births in Norway during that same time period and, of those eligible, 76% agreed to participate (N=763). After completion of data collection and linkage with the Medical Birth Registry, all identifiers were permanently stripped from the data set, with no opportunity for further follow-up.

Birthweight

Newborn birthweight was recorded by obstetricians or midwives in the delivery room or abstracted from obstetric records. We double checked and did not observe birthweight values +/- 5 SD from the mean.

Methylation measurements

Epigenome-wide DNA methylation was measured in 898 newborns, using DNA extracted from heel stick blood samples that were collected 2-3 days after delivery as part of a standardized program of testing for phenylketonuria (PKU). One microgram of DNA was bisulfite converted using the EZ DNA Methylation kit following the manufacturer's protocol. 898 newborn and 60 technical control samples were run on Illumina HumanMethylation450 BeadChips according to the manufacturer's instructions at the NIH Center for Inherited Disease Research. 22 samples having percentage of low quality data points (undetectable (detection $p > 0.05$) CpG or number of beads < 3) greater than 1% were excluded; 6 samples were excluded due to missing data for the covariates in the analysis models as required for the complete data analysis; total of 68 samples were excluded due to: 51 preterm births (gestational week <37), 11 non-singletons and 6 samples were both preterm birth and non-singleton. After exclusions, 802 samples remained for analysis.

Raw methylation intensity data were first background corrected using ENmix model*(70)*, and then Methylated (M) or Unmethylated (U) intensities were separately quantile normalized across samples for Infinium I or II probes. The β-value (M/(M+U+100)) was then adjusted for probe type bias using BMIQ method*(19)*.

Covariates

Information on maternal age, parity, and maternal education was collected in the same self-administered questionnaires (around 3-4 months after delivery). Facial cleft status was categorized as none (control), cleft lip with or without cleft palate, and cleft palate only. Analyses were additionally adjusted for a categorical variable kids birth year.

Cell type correction

We used the Houseman method*(6)* with the estimateCellCounts function in the Minfi package*(7)* in R*(8)* to estimate relative proportions of six white blood cell subtypes (CD4+ T-lymphocytes, CD8+ T-lymphocytes, NK (natural killer) cells, B-lymphocytes, monocytes and granulocytes).

Batch correction

We additionally corrected the analyses for batch effect by including bisulfite plate variable.

Exclusion criteria

For the main analysis we excluded n=68 participants; 57 infants born preterm (<37 weeks), and non-singleton infants (n=11). This information was obtained from questionnaires completed by the mothers shortly after delivery and combined with medical birth records.

The Newborn Epigenetics Study (NEST)

Design and study population

The Newborn Epigenetics STudy (NEST) is a multiethnic birth cohort designed to identify the effects of early exposures on epigenetic profiles and phenotypic outcomes. Pregnant women were recruited from prenatal clinics serving Duke University

Hospital and Durham Regional Hospital Obstetrics facilities in Durham, North Carolina from April 2005 to July 2009. Gestational age at enrollment ranged from 6 to 42 weeks (median 30 weeks). Eligibility criteria were women aged 18 years or older, English speaking, pregnant, and an intention to use one of the two obstetrics facilities. Among these, women infected with HIV or intending to give up custody of the offspring of index pregnancy were excluded. Current smokers were targeted for the first ~200 participants. Of the 1101 women who met eligibility criteria and were approached, 895 (81%) were enrolled and umbilical cord blood was collected from 741 infants. The current analysis was limited to the 413 infants with 450k and covariate data. This study was approved by the Duke Institutional Review Board. Additional details about NEST may be found in previous publications*(71, 72)*.

Birthweight

Birthweight was recorded by the clinician responsible for the delivery of the baby and abstracted from the medical records by the research team. Values +/- 5 SD from the mean were not found in the data.

Methylation measurements

Genomic DNA from buffy coat specimens was extracted from umbilical cord blood using Puregene Reagents (Qiagen, Valencia, CA). Bisulfite conversion was performed using the EZ-96 DNA Methylation Kit (Zymo Research Corporation) and DNA methylation was measured at 485,577 CpGs using Illumina Infinium HumanMethylation450 BeadChip (Illumina Inc., San Diego, USA). Illumina's GenomeStudio Methylation module version 1.0 (Illumina Inc.) was used to calculate the methylation level at each CpG as the beta value. Probe and sample-specific quality control was performed in the NEST cohort using a similar approach to MoBa1 and MoBa2 cohorts. Specifically, control probes (N=65) and probes on X (N=11 230) and Y (N=416) chromosomes were excluded as well as CpGs missing > 10% of methylation data. Samples indicated by Illumina to have failed or have an average detection P-value across all probes < 0.05 and samples with gender mismatch were also removed. The two different probe designs by applying the intra-array normalization strategy Beta Mixture Quantile dilation (BMIQ)*(19)*.

Covariates

The gestational age and gender of the child were collected from medical records following delivery. Maternal smoking status, socioeconomic status (education), age, prepregnancy body mass index (BMI), parity, and race were reported by the mother on a questionnaire completed during pregnancy. Models were stratified by race/ethnicity group (Caucasian or African American).

Cell type correction

The Reinius-based Houseman method*(5, 6)* was used with the estimateCellCounts function in the Minfi package*(7)* in R*(8)* to estimate relative proportions of six white blood cell subtypes (CD4+ T-lymphocytes, CD8+ T-lymphocytes, NK (natural killer) cells, B-lymphocytes, monocytes and granulocytes).

Batch correction

The Empirical Bayes method via ComBat was applied for batch correction using the sva package in R*(16)*.

Exclusion criteria

For the main analysis we excluded n=217 participants: 90 infants born preterm (<37 weeks), non-singleton births (n=3), missing covariates (n=54) and whose mothers experienced pre-eclampsia (n=24) or diabetes (n=29) during pregnancy or were a race other than Caucasian or African-American (n = 17). This information was obtained from questionnaires completed by the mothers shortly after delivery and combined with medical birth records.

The Northern Finland Birth Cohorts (NFBC1966 and NFBC1986)

Design and study population

NFBC1966: The Northern Finland Birth Cohort 1966 is a prospective follow-up study of children from the two northernmost provinces of Finland*(73)*. 96% of all woman in this region with expected delivery dates in 1966 were recruited though maternity health centres (12,058 live births). All individuals still living in northern Finland or the Helsinki area (n = 8,463) were contacted and invited for clinical examination. A total of 6007 participants attended the clinical examination at the participants' age of 31 years. DNA was extracted from blood samples given at the clinical examination (5,753 samples available)*(74)*.The subset with DNA is representative of the original cohort in terms the major environmental and social factors known to influence the tested trait. An informed consent for the use of the data including DNA was obtained from all subjects. DNA methylation was measured for 807 randomly selected subjects that attended the clinical examination and completed the questionnaire For DNA methylation marker calling we used a detection P-values threshold of <10-16 A call rate filter of 95% was applied to the all autosomal Illumina probes yielding 459378 probes for association testing. 67 samples were excluded due to low marker call rate (<95%). 7 samples were excluded for gender inconsistency, one sample for globally outlying DNA methylation values (1st PC score of the DNA methylation values outside mean +/- 4SD).

NFBC1986: The Northern Finland Birth Cohort 1986 consists of 99% of all children, who were born in the provinces of Oulu and Lapland in Northern Finland between 1 July 1985 and 30 June 1986. 9,203 live-born individuals entered the study*(75)*. At the age of 16, the subjects living in the original target area or in the capital area (n=9,215) were invited to participate in a follow-up study

including a clinical examination. 7344 participants attend the study in year 2001/2002, of which 5654 completed the postal questionnaire, the clinical examination and provided a blood sample*(76)*. DNA was extracted from all 5654 blood samples. An informed consent for the use of the data including DNA was obtained from all subjects. DNA methylation was recoded on Illumina HumanMethlation450K array for 566 randomly selected subjects. 24 technical replicates were excluded. 18 samples did not reach a call rate of >95% applying a detection P-value filter of 10^{-16}. We excluded 7 samples with gender inconsistency, no sample was outlying from the overall data structure (1st PC score of the DNA methylation values outside mean +/- 4SD). DNA methylation data of 517 samples with 466290 autosomal probes (call rate filter 95%) each were used for this analysis.

Birthweight
New-born birthweight was recorded by obstetricians or midwives. We did not observe birthweight values +/- 5 SD from the mean.

Methylation measurements
Methylation of genomic DNA was quantified using the Illumina HumanMethylation450 array according to manufacturer's instructions. Bisulfite conversion of genomic DNA was performed using the EZ DNA methylation kit according to manufacturer's instructions (Zymo Research, Orange, CA).

Covariates
Data on maternal parity, socio-economic status, smoking, pre-pregnancy body mass index, age at birth, newborn gender and gestational age at birth were self-reported in questionnaires during pregnancy.

Cell type correction
Potential confounding effects of blood cell subtypes were estimated by the Houseman method*(6)*.

Batch correction
To account for batch effects in the data, beta values underwent a functional normalization approach described by Fortin *et al.(18)* using the first 10 PCs of the Illumina 450K array control probes. This approach includes subset quantile normalization of the data and normal-exponential out-of-band background correction.

Exclusion criteria
We excluded preterm born children (n=24 in NFBC66; n=12 in NFBC86) and children of mothers diagnosed with gestational diabetes cases (n=2 in NFBC66; n=4 in NFBC86) from the analysis.

New Hampshire Birth Cohort Study (NHBCS)
Design and study population
The New Hampshire Birth Cohort Study (NHBCS), which began in 2009, is an ongoing prospective study of over 1500 women receiving prenatal care in New Hampshire, USA*(77)*. Pregnant mothers were recruited between 24-28 weeks gestation. Eligibility criteria included literacy in English, between 18–45 years of age, reported use of a private well as the primary source of home drinking water, and singleton pregnancies. All participants provided written informed consent in accordance with the requirements of the Institutional Review Board (IRB) at Dartmouth College. Pre- and post-delivery questionnaires were administered to collect self-reported sociodemographic, lifestyle, and medical history data, and a structured medical records review was employed to collect information about the pregnancy and delivery. Cord blood samples are collected on ~80% of eligible deliveries.

Birthweight
Participants also consented to a medical record review, which allowed additional information to be recorded about prenatal infections, medication use, birth outcomes and delivery details, and general health of the women and their infants after birth.

Methylation measurements
This study consisted of the first participants born in the study with available cord blood samples for DNA methylation analysis, complete information for all covariates (n=96). DNA was bisulfite converted using the EZ DNA Methylation kit and subsequently subjected to epigenome-wide DNA methylation assessment using the Illumina Infinium HumanMethylation450 BeadChip at the University of Minnesota Genomics Core Facility following standardized protocols. Post-array processing was conducted in the 'minfi' package in R. Array control probes were used to assess the quality of our samples and evaluate potential poor bisulfite conversion or color-specific issues for each array. Probes with detection p-values > 0.01 in at least one sample were removed. Data was then normalized using functional normalization (funNorm).

Covariates
Self-reported smoking during pregnancy (any smoking during pregnancy vs. no smoking during pregnancy), maternal highest educational attainment (high school graduation or less vs. at least some post high school education), parity (ever previously gave birth vs. never previously gave birth), and maternal age (continuous) were collected via questionnaire. Infant sex and gestational age were collected via medical records abstraction.

Cell type correction

Proportions of cell types were estimated from the 450K DNAM via the estimateCellCounts function*(5, 6)* within the 'minfi' package in R. Due to the sum of the six estimated cell proportions adding to 1.0 for each participant, only five of the cell-types were added to the models as covariates (granulocytes were excluded).

Batch correction

Batch effects were removed from the methylation data via ComBat; removal of batch effects was confirmed with principal components analysis.

Exclusion criteria

For the main analysis we excluded n=19 participants: 7 infants born preterm (<37 weeks), and whose mothers experienced pre-eclampsia (n=1) or diabetes (n=11) during pregnancy. This information was obtained from medical birth records.

Netherlands Twin Register (NTR)

Design and study population

The subjects were twins who participated in longitudinal survey studies from the Netherlands Twin Register (NTR)*(78)* and participated in the NTR biobank project between 2004 and 2011*(79)*. The NTR is a longitudinal twin-family study with no other selection criteria than being a multiple or one of their family members. Longitudinal surveys have been described in detail previously*(78)*. Complete information (DNA methylation, birthweight and all covariates from model 9) were available for 175 samples from 168 individuals who met the inclusion criteria. For 7 individuals, longitudinal methylation data (two samples) were included in the EWAS. Informed consent was obtained from all participants. The study was approved by the Central Ethics Committee on Research Involving Human Subjects of the VU University Medical Centre, Amsterdam, an Institutional Review Board certified by the U.S. Office of Human Research Protections (IRB number IRB00002991 under Federal-wide Assurance-FWA00017598; IRB/institute codes, NTR 03-180).

Birthweight

Data on birthweight were obtained from self-report by the twins themselves or their parents. Data collected across multiple surveys and projects were combined and consistency across family members and time was checked. When multiple data points differed by less than 200 grams, the average was taken, and in the cases of larger differences, data were excluded. In accordance to the PACE analysis plan, individuals with a birthweight lower than 2500 grams were excluded. After this exclusion, birthweight values > 5SD above the mean or lower than 5 SD below the mean did not occur in this dataset. Only the continuous birthweight analyses were performed. The dichotomous analysis (high (>4000g) versus normal (2500-4000g)) was not performed because few very twins had a birthweight greater than 4000g.

Methylation measurements

Blood sampling procedures have been described in detail previously*(79)*. DNA methylation was assessed with the Infinium HumanMethylation450 BeadChip Kit (Illumina, San Diego, CA, USA Illumina, San Diego, CA, USA) by the Human Genotyping facility (HugeF) of ErasmusMC, the Netherlands (http://www.glimdna.org/) as part of the Biobank-based Integrative Omics Study (BIOS) consortium*(80)*. DNA methylation measurements have been described previously*(80, 81)*. Genomic DNA (500ng) from whole blood was bisulfite treated using the Zymo EZ DNA Methylation kit (Zymo Research Corp, Irvine, CA, USA), and 4 µl of bisulfite-converted DNA was measured on the Illumina 450k array following the manufacturer's protocol. A number of sample- and probe-level quality checks and sample identity checks were performed (described in detail previously*(81)*). In short, sample-level QC was performed using MethylAid*(82)*. Probes were set to missing in a sample if they had an intensity value of exactly zero, or a detection $p>.01$, or a bead count of<3. After these steps, probes that failed based on the above criteria in >5% of the samples were excluded from all samples (only probes with a success rate≥ 0.95 were retained). Probes were also excluded from all samples if they mapped to multiple locations in the genome*(18)*. The methylation data were normalized with functional normalization*(6)* and normalized intensity values were converted into beta (β)-values. In accordance to the PACE analysis plan, the methylation dataset was trimmed on: (25th percentile -3*IQR) and (75th percentile+3*IQR). In total, 453288 sites were analysed.

Covariates

The following covariates were included in model 9: gestational age, sex, maternal educational attainment, maternal smoking during pregnancy, maternal age, maternal BMI, older siblings (yes/no), array row number, 3 PCs based on genome-wide methylation data, Bcell, CD4T, CD8T, Gran, Mono, NK. The following covariates were included in model 11: gestational age, sex, maternal educational attainment, maternal smoking during pregnancy, maternal age, maternal BMI, older siblings (yes/no), array row number, 3 PCs based on genome-wide methylation data, Bcell, CD4T, CD8T, Gran, Mono, NK, age, BMI, smoking, educational attainment person. Information on BMI and smoking status were collected as part of the NTR biobank project at the moment of blood draw*(79)*. Data on gestational age, maternal age at the time of delivery, and maternal pre-pregnancy BMI were obtained retrospectively from self-report of the mother. Information on whether the twins had older siblings was obtained through self-report by the twins. Data on educational attainment and maternal educational attainment were obtained from self-report by the

twins themselves or their parents. Data on educational attainment that were collected across multiple surveys were combined and consistency across family members and time was checked. Educational attainment was defined as the highest completed level of education at the age of 25 or higher. We analysed information on maternal smoking during pregnancy that was obtained in NTR Survey 10 (data collection in 2013) with the following question: Did your mother ever smoke during pregnancy? with answer categories: no, yes, I don't know. For twin pairs, the answers were checked for consistency and missing data for one twin were supplemented with data from the co-twin where possible: If one twin answered "yes" or "no", and his/her co-twin did not fill out the survey, or did not answer this question, or answered "I don't know", the missing information was replaced by the information supplied by the co-twin. If one twin answered "yes" and the co-twin answered "no" (3.6% of twin pairs for which an answer was available for both twins), data from both twins were set to missing. Ethnicities was defined using genetic data, which was checked for consistency with self or maternal reports.

Cell type correction
The minfi package was used to calculate the cell proportions using the Houseman method*(6)*. These include: CD8T, CD4T, NK, Bcell, Mono and Gran.

Batch correction
Illumina 450k array row and 3 PCs based on the genome-wide methylation data were included as covariates to account for technical variability between samples, as these PCs were correlated with several indices related to the lab procedure, such as sample plate and order of processing, as described previously.

Exclusion criteria
In accordance to the PACE analysis plan, we excluded preterm births (i.e. ≥37 weeks only) and individuals with a birthweight lower than 2500 grams. Information on maternal diabetes and pre-eclampsia were not available for these subjects.

Prevention and Incidence of Asthma and Mite Allergy (PIAMA)
Design and study population
The PIAMA study is a birth cohort study of children born between 1996-1997. Details of the study design have been published previously*(83)*. In brief, pregnant women were recruited during their first trimester from the general population in 1996-1997 through antenatal clinics in the north, west and center of the Netherlands. Non-allergic pregnant women were invited to participate in a ''natural history'' study arm. Pregnant women identified as allergic through the screening questionnaire were allocated primarily to an intervention arm with a random subset allocated to the natural history arm. The intervention involved the use of mite-impermeable mattress and pillow covers. The study started with 3,963 newborns. Parents completed questionnaires on demographic factors, risk factors for asthma and respiratory symptoms at the child's age of 3 months, annually from 1 to 8 years of age, and at 11, 14, and 16 years of age. Clinical examinations were performed in subgroups at ages 4, 8, 12 and 16 years.

Birthweight
Parents were asked to report their child's birthweight, as stated in the delivery report, in the 3- months questionnaire.

Methylation measurements
In the PIAMA study, DNA from peripheral blood samples was extracted using the QIAamp blood kit (Qiagen or equivalent protocols), followed by precipitation-based concentration using GlycoBlue (Ambion). DNA concentration was determined by Nanodrop measurement and Picogreen quantification. 500 ng of DNA was bisulphite-converted using the EZ 96-DNA methylation kit (Zymo Research), following the manufacturer's standard protocol. After verification of the bisulphite conversion step using Sanger Sequencing, DNA concentration was normalized and the samples were randomized to avoid batch effects. The DNA methylation was measured using the Illumina Infinium HumanMethylation450 beadchip. Each chip included one control DNA sample for quality control purposes. Data preprocessing was performed using the Minfi package*(29)*. We implemented sample filtering to remove bad quality samples (call rate <99%). Moreover, we used 65 SNP probes to check for concordances between paired DNA samples. Paired samples from the same individuals which show Pearson correlation coefficient <0.9 were regarded as sample mixed ups and were excluded from the study. We further assessed the methylation distribution of the X-chromosome to verify gender. After QC, 207 good quality 8 years and 640 good quality 16 years PIAMA samples were used in the analysis. During processing, the probes on sex chromosomes, the probes that mapped to multiple loci, 65 SNP-probes and the probes containing SNPs at the target CpGs with a MAF>5% were excluded*(13)*. We implemented "DASEN"*(45)* to perform signal correction and normalization.

Covariates
Maternal age was defined as a continuous variable. Maternal social class was defined as the highest attained educational level and coded in three categories: 1=primary school, lower vocational or lower secondary education (low) 2=intermediate vocational education or intermediate/higher secondary education (intermediate) 3= higher vocational education and university (high). Parity was defined as older siblings living in the PIAMA home. Pre-pregnancy maternal BMI was calculated using height and weight of

the mother before pregnancy, self-reported in the questionnaire when the child was 1 year of age. Maternal smoking during pregnancy was coded as 1= no smoking in pregnancy, 2=smoking but stopped in the first 16 weeks, 3=smoking for longer than 16 weeks. The current status of asthma was also added as co-variable since the samples were enriched with asthma cases.

Cell type correction
Cell type correction was applied using the reference-based Houseman method*(6)* in the Minfi package*(29)*. This method estimates the relative proportions of six white blood cell subtypes (CD4+ T-lymphocytes, CD8+ T-lymphocytes, NK (natural killer) cells, B-lymphocytes, monocytes and granulocytes).

Batch correction
In age 8 models, bisulfite conversion kits (2 batches) have been used as batch variables. In age 16 models, the samples were run into two batches, and therefore batch was included in the model.

Exclusion criteria
For the main analysis of age 8, we excluded n=10 participants due to low birthweight (<2, 500g). For the main analysis of age 16, we excluded 19 participants due to low birthweight (<2,500g).

Prediction and Prevention of Preeclampsia and Intrauterine Growth Restriction study (PREDO)
Design and study population
Data were from the Prediction and Prevention of Preeclampsia and Intrauterine Growth Restriction (PREDO) Study, which is a longitudinal multicenter pregnancy cohort study of Finnish women and their singleton children born alive between 2006-2010*(84)*. We recruited 1079 pregnant women, of whom 969 had one or more and 110 had none of the known risk factors for preeclampsia and intrauterine growth restriction. The recruitment took place in arrival order when these women attended the first ultrasound screening at 12+0-13+6 weeks+days of gestation in one of the ten hospital maternity clinics participating in the study. The cohort profile*(84)* contains details of the study design and inclusion criteria. The study protocol was approved by the Ethical Committees of the Helsinki and Uusimaa Hospital District and by the participating hospitals. A written informed consent was obtained from all women. The study has been registered as ClinicalTrials.gov identifier ISRCTN14030412.

Birthweight
Newborn birthweight was measured by obstetricians or midwives in the delivery room and abstracted from birth records. We double checked and did not observe birthweight values +/- 5 SD from the mean.

Methylation measurements
Cord blood samples were ran on Illumina 450K Methylation arrays. To limit batch effects, we randomized all samples over the 96-well plates, based on gender and maternal risk factors for pre-eclampsia. Samples were placed on 96-well plates. Bisulfite conversion was performed using the EZ-96 DNA methylation kit (Zymo research Corporation, Irvine, USA). Then we used the Infinium HumanMethylation450 BeadChip (Illumina Inc., San Diego, USA) to measure the methylation level as a beta value ranging from 0 (no methylation) to 1 (complete methylation).The quality control pipeline was set up using the R-package minfi*(8, 29)*. Three IDs were excluded as they were outliers in the median intensities. Furthermore, 20 IDs showed disconcordance between phenotypic sex and estimated sex and were excluded. Nine IDs were contaminated with maternal DNA (Morin et al, submitted) and were also removed. Methylation beta-values were normalized using the funnorm function. We excluded any probes on chromosome X or Y, probes containing SNPs and cross-hybridizing probes according to Chen *et al*.*(13)* and Price *et al*.*(49)* Furthermore, any CgGs with a detection p-value > 0.01 in at least 25% of the samples were excluded.

Covariates
Data on maternal parity, socio-economic status and smoking during pregnancy, pre-pregnancy body mass index, age at childbirth, newborn sex and gestational age at birth were extracted from the Finnish Medical Birth Register.

Cell type correction
We used the method by Houseman*(6)* with the estimateCellCounts function in the Minfi package in R to estimate relative proportions of six white blood cell subtypes (CD4+ T-lymphocytes, CD8+ T-lymphocytes, NK (natural killer) cells, B-lymphocytes, monocytes and granulocytes).

Batch correction
After normalization two batches, i.e. slide and well, were significantly associated and were removed iteratively using the Combat method*(16)*.

Exclusion criteria
For the main analysis we excluded n=254 participants: 33 infants born preterm (<37 weeks), and whose mothers experienced pre-eclampsia (n=62) or diabetes (n=192) during pregnancy. This information was obtained from questionnaires completed by the mothers shortly after delivery and combined with medical birth records.

Programming of Intergenerational Stress Mechanisms (PRISM)

Design and study population

The PRogramming of Intergenerational Stress Mechanisms (PRISM) study, is a prospective pregnancy cohort of mother-child pairs originally designed to examine how perinatal stress influences respiratory health in children. Women were recruited from prenatal clinics during the first or second trimester (<28 weeks gestation) from the Beth Israel Deaconess Medical Center (BIDMC) and the East Boston Neighborhood Health Center in Boston Massachusetts, USA, from March 2011 to August 2012 and from the Icahn School of Medicine at Mount Sinai in New York, New York, USA, starting in April 2013 where collections are still underway. Recruitment sites were chosen to ensure desired heterogeneity in sociodemographic and racial/ethnic characteristics. Eligibility criteria included: (i) English- or Spanish-speaking; (ii) age ≥18 years at enrollment; and (iii) singleton pregnancy. Procedures were approved by the Institutional Review Boards at the Brigham and Women's Hospital (BWH) and the Icahn School of Medicine at Mount Sinai. Beth Israel Deaconess Medical Center (BIDMC) relied on BWH for review and oversight of the protocol. Written consent was obtained from all participants. Additional details about the cohort and methylation measures have been described elsewhere*(85, 86)*. PRISM consisted of 41.3% white European, 38.4% African American, 10.1% Hispanic and 10.1% other ancestries.

Birthweight

Newborn birthweight was abstracted from obstetric records. The distribution was checked for plausibility, with no values +/- 3 SD from the mean after restricting to the analytic dataset of full term births. Given the limited sample size, we treated birthweight as a continuous trait in grams.

Methylation measurements

Cord blood was collected prior to delivery of the placenta from all samples that could be retrieved during delivery. Cord blood was separated into plasma and buffy coat by centrifugation and buffy coat was stored at -20°C. DNA was isolated using Qiagen Tissue DNA extraction kits (Qiagen, Valencia CA) and quantified using an Implen Nanophotometer Pearl (Westlake Village, CA). 500ng of DNA was bisulfite treated using the EZ DNA Methylation-Gold™ Kit (Zymo Research, Orange, CA) and analyzed by the Illumina Infinium HumanMethylation450 BeadChip array. Samples were arranged on chips and plates with a stratified randomization followed by statistical checks for balance on birthweight z-score, gestational age, sex, and city of collection. The presence of failed arrays or outliers was checked with detection p-values (all samples passed with detection p-values <0.05 in >99% of probes) and through visualization of principal components analysis (PCA). Potential batch effects were further assessed with five pairs of technical replicates per tissue that were arranged across chips and plates. Sample identity was checked via imputed sex and agreement of genotype with paired tissues. Probes with detection p-values >0.05 or beadcount <3 in >1% of samples (a total of 1217 probes) were dropped using the wateRmelon pfilter function resulting in 484,360 remaining probes*(45)*. Data were preprocessed using background correction*(25)*, dye bias and probe type adjustment*(19)*. BMIQ (Beta Mixture Quantile dilation) intra-sample normalization was applied to all probes to adjust the methylation values of Infinium II probes into a statistical distribution characteristic of Infinium I probes.

Covariates

Data on maternal primiparity (dichotomous), education (categorical with five levels) as a proxy for socio-economic status, any smoking during pregnancy (dichotomized), pre-pregnancy body mass index, and maternal age were self-reported in questionnaires administered during the second or third trimester of pregnancy. Newborn sex and gestational age at birth were abstracted from the medical record.

Cell type correction

We used the Reinius-based Houseman method*(5, 6)* with the estimateCellCounts function in the Minfi package [8] in R [9] to estimate relative proportions of six white blood cell subtypes (CD4+ T-lymphocytes, CD8+ T-lymphocytes, NK (natural killer) cells, B-lymphocytes, monocytes and granulocytes).

Batch correction

We additionally corrected the analyses for batch effect by including the 96-well bisulfite conversion plate (laboratory batch) as a covariate (categorical variable with seven levels).

Exclusion criteria

From the main analysis we excluded 10 participants because of gestational diabetes, 9 participants because of gestational hypertension and 10 because pre-term borns.

Programming Research in Obesity, GRowth Environment and Social Stress (PROGRESS)

Design and study population

Programming Research in Obesity, GRowth Environment and Social Stress (PROGRESS) is a prospective birth cohort of healthy pregnant women. We recruited women if they were less than 20 weeks of gestation through the Mexican Social Security System between July 2007 and February 2011. We enrolled women greater than 18 years old that have access to a telephone and a plan to reside within Mexico City for the following 3 years. We excluded people with diagnosis of heart or kidney disease, use of steroids

or anti-epilepsy drugs and daily alcohol consumption. Women provided written informed consent for themselves and their children.

Birthweight
Birth length and weight were recorded at delivery or abstracted from the infants' hospital chart. At later study visits, length/height and weight were measured using the Infant-o-meter (Health O Meter Inc.) for children 1-12 months old and the Health-o-meter (Health O Meter Inc.) for children older than 12 months. For the analysis model we treated birthweight as a continuous variable in grams.

Methylation measurements
We collected umbilical cord venous blood at the time of delivery and stored whole blood in PAXgene™ (PreAnalytiX GmbH, Hombrechtikon Switzerland) tubes and extracted DNA using PAXgene kits. Umbilical cord blood samples were aliquoted and frozen until manual DNA extraction including a red blood cell lysis step followed by isopropanol and ethanol extraction of DNA from total white blood cells. Resulting DNA samples were randomized for plating and bisulfite converted and analyzed on the Illumina 450k BeadChip by Illumina FastTrack Services (Illumina Inc., San Diego CA), prior to preprocessing and quality control with the methylumi package.

Covariates
Major covariates include maternal pre-pregnancy BMI (calculated as BMI during 2nd trimester minus 2 kg/m2), parity, socio-economic status, smoking, age at birth and new born gender were obtained from baseline questionnaires, hospital records or field measurements. Gestational age was based on the difference between the birth date and the mother's report on enrolment of her last menstrual period.

Cell type correction
We estimated the proportions of the six white blood cell subtypes: CD4+ T-lymphocytes, CD8+ T-lymphocytes, NK (natural killer) cells, B-lymphocytes, monocytes and granulocytes, using the Reinius-based Houseman method with the *estimateCellCounts* function in the Minfi package of R.

Batch correction
We additionally corrected the analyses for batch effect by including the Illumina Infinium HumanMethylation450 BeadChip number.

Exclusion criteria
For the main analysis we excluded infants born preterm (<37 weeks, n=118), which automatically excluded the participants whose mothers experienced pre-eclampsia and hypertension during the pregnancy. This information was obtained from the mothers' questionnaires completed shortly after the delivery and combined with medical birth records.

Project Viva

Design and study population
Project Viva is a population-based prospective birth cohort in Eastern Massachusetts. Pregnant women at <22 weeks gestation with the ability to answer questions in English and singleton pregnancies were recruited at their first prenatal visit at a participating obstetric office from 1999 to 2002. Of 2218 live births, 1018 cord blood samples were collected. All women gave written informed consent for the study, and 507 mothers additionally gave written informed genetic consent. Institutional review boards at all participating institutions gave approval for this study*(87)*.

Birthweight
Newborn birthweight in grams was obtained from hospital medical records. We did not observe birthweight values +/- 5 SD from the mean.

Methylation measurements
For the 507 samples that gave genetic consent, DNA was extracted from cord blood using the Qiagen Puregene Kit (Valencia, CA) and sodium bisulphate converted using the EZ DNA Methylation-Gold Kit (Zymo Research, Irvine, CA). Methylation beta values (0=no methylation, 1=complete methylation) were measured using the Infinium HumanMethylation450 BeadChip (Illumina Inc., San Diego, USA). During sample quality control, 22 samples were removed due to genotype mismatch, sex mismatch, and low quality, leaving 485 samples. After performing background correction with the normal-exponential out-of-band (noob)*(25)* method with dye-bias correction, we adjusted for probe type using Beta-Mixture Quantile dilation (BMIQ)*(19)*. We removed 3091 non-CG probes, 11,648 sex chromosome probes, and 459 probes with detection p-value<0.05 in less than 75% of samples, leaving 470,411 probes for analysis.

Covariates
Data on maternal parity, maternal education (as a measure of socio-economic status), smoking, pre-pregnancy body mass index, maternal age at enrollment, newborn gender and gestational age at birth were collected via mailed questionnaires and clinical records.

Cell type correction
We used the Reinius-based Houseman method*(5, 6)* with the *estimateCellCounts* function in the Minfi package*(7)* in R*(8)* to estimate relative proportions of six white blood cell subtypes (CD4+ T-lymphocytes, CD8+ T-lymphocytes, NK (natural killer) cells, B-lymphocytes, monocytes and granulocytes).

Batch correction
We additionally corrected the analyses for batch effect using ComBat*(16)*, using Sentrix position within each of two scanning batches as the batch variable. The batch variable had 19 unique levels.

Exclusion criteria
Because analyses were stratified by self-reported race and we had very few non-white children, we excluded children of non-white mothers (n=141) in our analysis. For the main analysis of white children, we excluded n=15 participants: 12 infants born preterm (<37 weeks), 2 infants whose mothers had pre-gestational diabetes, and 1 infant whose mother did not have a recorded pre-pregnancy BMI. This information was obtained from questionnaires completed by the mothers shortly after delivery and combined with medical birth records.

The West Australian Pregnancy Cohort (Raine)
Design and study population
The Western Australia Pregnancy Cohort (Raine) study (http://www.rainestudy.org.au) is a longitudinal Australian birth cohort that has serially assessed the offspring of 2900 pregnant women from 18 weeks gestation in utero. Follow-up of the offspring has been undertaken at 1, 2, 3, 5, 8, 10, 14, 17 and 24 years*(88, 89)*.

Birthweight
Birthweight was measured by hospital midwives immediately after birth on standardized hospital neonatal scales.

Methylation measurements
DNA was extracted from whole blood samples obtained at 17-year-old follow up. Bisulphite conversion was prepared from whole blood cells by standard phenol:chloroform extraction and ethanol precipitation. Processing of the Illumina Infinium HumanMethylation450 BeadChips was carried out by the Centre for Molecular Medicine and Therapeutics (CMMT) (http://www.cmmt.ubc.ca). The raw IDAT files were imported into R using the rnb.run.import() function available in the RnBeads package*(90)*. Two packages were used to perform quality control checks of the samples; shinyMethyl*(91)* and MethylAid*(82)*. Three samples were evident as outliers and removed. We removed intentional SNP probes (n=65), sex chromosome probes (n=11,648), and probes with a detection p-value greater than 0.05 in any sample (n=10,777). A further 160 probes with low bead counts (bead counts less than 3 in more than 5% of samples) were removed. Probes were normalized using BMIQ*(19)* and an additional 94 probes were removed resulting in 462,833 probes for analysis.

Covariates
Maternal BMI was calculated using self-reported pre-pregnancy weight and height. Data on maternal age, income, parity and maternal smoking during pregnancy were assessed by questionnaires at 18 and 34 weeks pregnancy. Maternal age at delivery was derived from the mother's report of her own and her baby's dates of birth. We used plate and plate position as technical batch variables. Gestational age was based on the date of the last menstrual period, unless there was discordance with ultrasound measurements at less than 18 weeks gestation. In those cases of discordance of more than 7 days, the estimate was based on ultrasound biometry at 18 weeks. First date of last menstrual period (LMP) was recorded by the mother at the 16-18-week survey. Weight was measured by Wedderburn Chair Scales to the nearest 100 grams and height to the nearest 0.1 cm using a Holtain Stadiometer, at the 17 year follow up. Body mass index (BMI) was calculated as weight in kilograms divided by height in metres squared. Current smoking status at 17 years of age was derived from an online confidential questionnaire answered by the adolescent, in response to the question "have you smoked in the last 4 weeks?"

Cell type correction
The relative proportions of CD8+ T-lymphocytes, CD4+ T-lymphocytes, NK cells, B-lymphocytes, monocytes and granulocytes were inferred using the estimateCellCounts function within minfi*(29)*, based on the Houseman method*(6)*.

Batch correction
Plate and row number were included in all models to adjust for potential batch effects.

Exclusion criteria
Individuals whose mother had maternal diabetes or pre-eclampsia were excluded, as well as multiple births and pre-term births.

Rhode Island Child Health Study (RICHS)

Design and study population

Study participants are part of the Rhode Island Child Health Study (RICHS), which enrolled 840 mother-infant pairs following delivery at Women and Infants Hospital (Providence, RI, USA) from 2009-2014*(92)*. Study participants provided written informed consent and all protocols were approved by the Institutional Review Boards at Women and Infants Hospital and Dartmouth College. Eligibility criteria included being between the ages of 18 and 40 years, free of life threatening conditions, no congenital or chromosomal abnormalities, and gestational time greater than 37 weeks. Infants were singleton births, with gestation to term (≥ 37 weeks). Term infants born small for gestational age (SGA, <10th percentile), or large for gestational age (LGA, >90th percentile), were oversampled, then infants appropriate for gestational age (AGA, ≥10th percentile and ≤90th percentile) matched on gender, gestational age (±3 days), and maternal age (±2 years) were also enrolled. Structured medical records review was performed to collect maternal inpatient information from the delivery as well as anthropometric and clinical data. After delivery but prior to discharge, an interviewer-administered questionnaire was used to obtain information on exposures, demographics, and lifestyle factors. Cord blood samples were obtained as residual tissues from samples collected at delivery and maintained in the Department of Pathology for potential clinical use. Once the infant was discharged, remaining cord blood samples were collected for the study. A total of 450 newborn cord blood samples were collected, and the major demographic features of the infants with cord blood samples did not differ from those of the whole cohort.

Birthweight

Participants also consented to a medical record review, which allowed for additional details on birth outcomes and delivery.

Methylation measurements

This study consisted of the first participants born in the study with available cord blood samples for DNA methylation analysis, complete information for all covariates (n=89). DNA was bisulfite converted using the EZ DNA Methylation kit and subsequently subjected to epigenome-wide DNA methylation assessment using the Illumina Infinium HumanMethylation450 BeadChip at the University of Minnesota Genomics Core Facility following standardized protocols. Post-array processing was conducted in the 'minfi' package in R. Array control probes were used to assess the quality of our samples and evaluate potential poor bisulfite conversion or color-specific issues for each array. Probes with detection p-values > 0.01 in at least one sample were removed. Data was then normalized using functional normalization (funNorm).

Covariates

Self-reported smoking during pregnancy (*any smoking during pregnancy vs. no smoking during pregnancy*), maternal highest educational attainment (*high school graduation or less vs. at least some post high school education*), parity (*ever previously gave birth vs. never previously gave birth*), and maternal age (*continuous*) were collected via questionnaire. Infant sex and gestational age were collected via medical records abstraction.

Cell type correction

Proportions of cell types were estimated from the 450K DNAM via the estimateCellCounts function*(5, 6)* within the 'minfi' package in R. Due to the sum of the six estimated cell proportions adding to 1.0 for each participant, only five of the cell-types were added to the models as covariates (granulocytes were excluded).

Batch correction

Batch effects were removed from the methylation data via ComBat; removal of batch effects was confirmed with principal components analysis.

Exclusion criteria

For the main analysis we excluded 7 participants whose mothers experienced diabetes during pregnancy. This information was obtained from questionnaires completed by the mothers shortly after delivery.

The Swedish Twin study On Prediction and Prevention of Asthma (STOPPA)

Design and study population

The Swedish Twin study On Prediction and Prevention of Asthma (STOPPA)*(93)* is a twin cohort study including 752 individuals. Twins 9-14 years of age were selected from an on-going data collection within the Child and Adolescent Twin study in Sweden (CATSS)*(94)* based on the pair's asthma status. Asthma concordant (ACC), asthma discordant (ADC) and healthy concordant (HCC) pairs were included and invited to take part in test centre visits including clinical examination, questionnaires, lung function testing and collection of biosamples. The study was approved by the regional ethical review board in Stockholm, Sweden. Written informed consent was collected from the study participants and their parents. Further details regarding STOPPA have been provided in a separate publication*(93)*. To allow for both twins from full pairs to be retained within the sample, generalized estimating equation (GEE) models were used in place of robust linear regression in STOPPA. By specifying twin pairs as clusters,

the GEE method produces robust standard errors and corrects for within-cluster (i.e. within-pair) correlations. The parameter estimates themselves are not affected. For these analyses the *drgee* R package was used*(95)*.

Birthweight

The study population has been linked to the Swedish population-based Medical Birth Register (MBR), from which birthweight was available from birth records. No values fell outside +/-5 SD. Because only one individual with birthweight >4000 grams remained within the study population following exclusions, only analyses featuring the continuous phenotype were performed using the STOPPA data.

Methylation measurements

STOPPA biosamples included whole blood samples from n=708 twins, i.e. all twins for whom blood sample collection was possible on the day of clinical examination. Following exclusions based on predefined criteria (n=339), missing data on covariates or outcomes (n=118), and sample quality (n=2), 249 twins remained for the current study. Of the 249 individuals with complete information, n=8 were below 10 (but above 9) years of age. Because this group was too small to run any analyses within separately, they were included in the adolescent group. DNA was extracted from whole blood (collected in a 4ml EDTA tube) using the Chemagic Star 400 kit (PerkinElmer chemagen, Baesweiler, Aachen, Germany) according to a standardized protocol. Samples were allocated between analysis plates and chips by complete randomization, with the exception that samples from twin pairs were kept within the same chip to allow for within-pair comparisons free of batch effects. Analyses were performed at the Mutation Analysis Facility (MAF) at Karolinska Institutet using the Infinium HumanMethylation450 Beadchip Kit (Illumina, Inc., San Diego, California, USA). Quality control, sample and probe filtering was performed using RnBeads*(90)*. Predicted gender and phenotype-based sex were compared, and matched for all samples. Probes overlapping with single nucleotide polymorphisms or specific nucleotide contexts (n=15,489), due to unreliable measurements (defined as detection p-values > 5*10-8, resulting in the filtering of n=3,663 probes and 2 samples), or which were located on sex chromosomes (n=10,942) were filtered out, leaving 455,483 CpG probes for the final analysis. The data set was normalized using the dasen method*(45)*.

Covariates

Gestational age, child's sex, maternal smoking during pregnancy (any/never), maternal BMI (calculated from weight and height at the first antenatal care visit), and parity were available from maternal antenatal and birth records (MBR). Maternal social class was defined by the highest self-reported education level achieved by the mother at the time of clinical examination, and categorized as "Less than high school level", "High school or equivalent degree", "Some college or university studies" or "3+ years of college/university or a university degree." Because recruitment to STOPPA was based on asthma case/control status within twin pairs, the case/control variable for asthma status within the individual was included as a covariate. Of the final 249 individuals, 114 (45.8%) had been recruited as asthma cases and 135 (54.2%) as controls. Study participants' own current smoking at the time of data collection had not been assessed and was therefore not included in the STOPPA sensitivity model.

Cell type correction

The relative proportions of CD8+ T-lymphocytes, CD4+ T-lymphocytes, NK cells, B-lymphocytes, monocytes and granulocytes were inferred using the estimateCellCounts function within minfi*(29)*, based on the Houseman method*(6)*.

Batch correction

Plate number was included in all models to adjust for potential batch effects.

Exclusion criteria

For the main analysis we excluded n=339 participants: 323 infants born preterm (<37 weeks), and whose mothers experienced pre-eclampsia (n=24) or diabetes (n=2) during pregnancy. This information was obtained from medical birth records.

The Gambia

Design and study population

The data was collected as part of a study in The Gambia (in Sub-Saharan West Africa) identifying biomarkers and understanding mechanisms for the relationship between aflatoxin exposure and child stunting. 251 whole blood samples (3 ml) were collected from children aged 2 years as part of the Early Nutrition and Immune Development (ENID) Trial*(96)*. For DNA extraction the blood was centrifuged, and the plasma was removed; DNA was then extracted from the resulting cell pellet using a salting out technique. For RNA extraction, a sample of whole blood was taken prior to centrifugation and stored in RNALater. All ethical approvals were obtained from the joint Gambia Government/MRC Unit, The Gambia Ethics Committee, and full informed consent was obtained from all participating families, prior to enrolment into the study. Of the samples collected, 243 samples had DNA methylation data (HM450), of which 84 samples also had expression data (HumanHT-12 v4 Expression BeadChip); both datasets were generated at the Epigenetics Group, International Agency for Research on Cancer, Lyon, France.

Birthweight

Birthweight was measured within 72 hours of delivery by a trained member of the study team, using a digital infant scale (Seca 334) and to the nearest 10g. We did not observe birthweight values +/- 5 SD from the mean. For the main analysis model, we treated birthweight as a continuous trait in grams, and, for the dichotomous model, we categorised birthweight in high (>4000g) versus normal (2500-4000g) birthweight.

Methylation measurements

Within The Gambia birth cohort, we selected 243 infants for the methylation study. From these infants, peripheral blood DNA was used for the epigenome-wide DNA methylation analyses. Bisulfite conversion (600 ng DNA/sample) was performed using the EZ-96 DNA methylation kit (Zymo research Corporation, Irvine, USA). Then the Infinium HumanMethylation450 BeadChip (Illumina Inc., San Diego, USA) was used to measure the methylation level as a beta value ranging from 0 (no methylation) to 1 (complete methylation). During the quality control, the binomial distribution was evident in all samples and coherent across samples, and we observed no gender mismatches, which we confirmed using the getSex function. We performed Functional normalization (FunNorm)*(18)* from minfi R package*(29)* to remove unwanted variation by regressing out variability explained by the control probes present on the array. We filtered out cross-reactive probes and probes on X or Y chromosomes, resulting in 470,964 remaining CpGs. We have added the ethnicity covariable for our specific analysis because our data comprises several ethnicities which we reclassified in 2 groups: Mandinka (n = 189) and other (n = 23).

Covariates

The covariates analysed were maternal parity, socio-economic status, smoking, pre-pregnancy body mass index, age at birth, newborn gender, gestational age at birth and ethnicity.

Cell type correction

We used the Reinius-based Houseman method*(5, 6)* with the *estimateCellCounts* function in the Minfi package*(29)* in R to estimate relative proportions of six white blood cell subtypes (CD4+ T-lymphocytes, CD8+ T-lymphocytes, NK (natural killer) cells, B-lymphocytes, monocytes and granulocytes).

Batch correction

We additionally corrected the analyses for batch effect using surrogate variable analysis (SVA)*(30)*.

Exclusion criteria

In the methylation subset of The Gambia cohort we excluded infants born preterm (<37 weeks) (n=2), and samples that didn't have complete data for covariates (n=29). Data on maternal diabetes and pre-eclampsia was not available in this cohort.

Gene expression analysis

The Gambia gene expression data was collected at 2 years of age. Whole blood was collected in Gambia and shipped to IARC, France, on dry ice where they were stored at -80 C. Samples were later thawed and extracted using RiboPure kit from Ambion (RNA purification kit for blood). All samples had a RIN greater than the acceptable threshold of 6.5 (range = 6.5 – 9.8; median = 9.1, mean ± SD = 9.0 ± 0.7). The high median and mean were in line with the observation that the vast majority of RNA samples had a RIN ~9.0, so we did not adjust for RNA quality. Gene expression data was generated using IlluminaHumanHT-12 v4 Expression BeadChip Kit at IARC, Lyon, France. Gene expression was normalized with the neqc R package, and expression transcripts were annotated using HumanHT-12 v4.0 Manifest File. Out of 104 samples that had expression data, 20 were excluded because they did not have methylation data. The final sample size was 84.

DNA methylation, DNA extraction and preprocessing has been described in the cohort-specific methods above. In brief, data quality was inspected using boxplots for the distribution of methylated and unmethylated signals, and inter-sample relationship (including gender mismatches) using multidimensional scaling plots and unsupervised clustering. QQ-plots of p values were used to rule out potential data inflation after statistical analyses. The remaining dataset was normalized using the funnorm normalization*(18)* of the minfi package*(29)*. Batch effects were then corrected with surrogate variable analysis (7 surrogate variables identified and adjusted for), and the effect of sex and blood cell proportions estimates were controlled out in a linear regression model. Cell proportions were estimated using the Houseman method*(6)*.

Only transcripts inside a 500kb window of a selected CpG (250 kb downstream and 250 kb upstream) were considered in the analysis. The model was controlled for technical and unwanted biological variation when estimating gene expression residuals, 8 surrogate variables were detected by surrogate variable analysis and added to a second model including sex and cell estimates.

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