

Supplementary Material

Prenatal alcohol exposure is associated with adverse cognitive effects and distinct whole-genome DNA methylation patterns in primary school children

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1 Supplementary Figures and Tables

1.1 Tables

Table S1: Differentially methylated CpGs with a p-value $< 10^{-3}$. All hits have been considered for functional clustering (Table 2). No multiple testing was applied due to 450k/genome-wide Bonferroni threshold of 10^{-7} .

| | Gene name | CpG | p-Value |
|----|-----------|------------|----------|
| 1 | TTBK2 | cg08194650 | 7.14E-06 |
| 2 | TARM1 | cg21966754 | 8.42E-06 |
| 3 | EMX1 | cg17090593 | 9.59E-06 |
| 4 | C1orf14 | cg22450662 | 1.13E-05 |
| 5 | ZNF706 | cg16269364 | 2.02E-05 |
| 6 | INPP5E | cg13741927 | 2.29E-05 |
| 7 | SNRNP27 | cg20337934 | 2.40E-05 |
| 8 | RAB40B | cg15250633 | 2.51E-05 |
| 9 | PARK2 | cg26872907 | 2.60E-05 |
| 10 | RASA3 | cg09634726 | 3.02E-05 |
| 11 | C6orf106 | cg10519906 | 3.24E-05 |
| 12 | PIK3CD | cg04287259 | 3.41E-05 |
| 13 | ATXN7L1 | cg03199646 | 3.82E-05 |
| 14 | KCNG2 | cg17174613 | 3.97E-05 |
| 15 | SMAD3 | cg01624571 | 4.24E-05 |
| 16 | TSSC4 | cg23594951 | 4.33E-05 |
| 17 | MRPS25 | cg18017531 | 4.60E-05 |

| | | | |
|----|------------|------------|----------|
| 18 | MRC2 | cg08863844 | 4.62E-05 |
| 19 | GTF2E2 | cg18356330 | 4.77E-05 |
| 20 | DPP6 | cg18239417 | 4.80E-05 |
| 21 | AATK | cg02460935 | 5.10E-05 |
| 22 | CHRNA5 | cg16751781 | 6.45E-05 |
| 23 | CASZ1 | cg16926809 | 6.91E-05 |
| 24 | CLDN16 | cg12982543 | 7.10E-05 |
| 25 | MT1F | cg02527372 | 8.31E-05 |
| 26 | PRDM16 | cg26014413 | 8.93E-05 |
| 27 | LSAMP | cg26033586 | 1.06E-04 |
| 28 | PID1 | cg17804911 | 1.07E-04 |
| 29 | SIPA1L3 | cg00344482 | 1.12E-04 |
| 30 | ZNF253 | cg26933068 | 1.12E-04 |
| 31 | MMEL1 | cg20776977 | 1.17E-04 |
| 32 | SBNO2 | cg00467278 | 1.27E-04 |
| 33 | ITGB2 | cg14112356 | 1.38E-04 |
| 34 | C17orf62 | cg14231704 | 1.43E-04 |
| 35 | ZMYND15 | cg01840020 | 1.46E-04 |
| 36 | EPB41L3 | cg16304950 | 1.48E-04 |
| 37 | LOC494141 | cg26558664 | 1.48E-04 |
| 38 | KPNA6 | cg10332202 | 1.59E-04 |
| 39 | SSU72 | cg10332202 | 1.59E-04 |
| 40 | POFUT2 | cg03515060 | 1.63E-04 |
| 41 | DYSF | cg08972867 | 1.63E-04 |
| 42 | MMRN2 | cg15347156 | 1.64E-04 |
| 43 | MAD1L1 | cg17815546 | 1.75E-04 |
| 44 | AK3 | cg14288486 | 1.76E-04 |
| 45 | RTN4IP1 | cg05080777 | 1.90E-04 |
| 46 | GABRB2 | cg01138867 | 1.94E-04 |
| 47 | SPAG1 | cg04958845 | 2.14E-04 |
| 48 | HLCS | cg16059256 | 2.14E-04 |
| 49 | SNORD115-7 | cg23841760 | 2.18E-04 |
| 50 | UBE2J2 | cg22670128 | 2.22E-04 |
| 51 | ATL3 | cg14589563 | 2.24E-04 |
| 52 | NACC2 | cg14350701 | 2.25E-04 |
| 53 | CADM1 | cg01850954 | 2.25E-04 |
| 54 | COL11A2 | cg20901684 | 2.25E-04 |
| 55 | MLKL | cg27459981 | 2.32E-04 |
| 56 | MSLN | cg06630425 | 2.39E-04 |
| 57 | FOXP1 | cg20088477 | 2.42E-04 |
| 58 | ZFP41 | cg06422464 | 2.46E-04 |
| 59 | C14orf86 | cg04836972 | 2.54E-04 |
| 60 | FAM172A | cg25015043 | 2.58E-04 |
| 61 | CNR1 | cg19961480 | 2.60E-04 |

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|-----|-------------|---------------|----------|
| 62 | SSB | cg14157244 | 2.67E-04 |
| 63 | MBD2 | cg16200116 | 2.78E-04 |
| 64 | RPH3AL | cg27489009 | 2.91E-04 |
| 65 | KCTD15 | cg20638467 | 2.93E-04 |
| 66 | PRDM12 | cg15700165 | 2.95E-04 |
| 67 | UNC5B | cg18858343 | 3.05E-04 |
| 68 | C17orf95 | cg02318866 | 3.15E-04 |
| 69 | AHNAK | cg20518446 | 3.16E-04 |
| 70 | GPR142 | cg20861314 | 3.19E-04 |
| 71 | GTF3C1 | cg00092389 | 3.21E-04 |
| 72 | AHNAK2 | cg13599499 | 3.32E-04 |
| 73 | HEATR1 | cg07700062 | 3.35E-04 |
| 74 | CASP10 | cg07754492 | 3.46E-04 |
| 75 | GBP4 | cg27285720 | 3.66E-04 |
| 76 | RPH3AL | cg18771195 | 3.70E-04 |
| 77 | INTS1 | cg15976779 | 3.71E-04 |
| 78 | NWD1 | cg09500002 | 3.74E-04 |
| 79 | SFXN3 | cg07372974 | 3.78E-04 |
| 80 | RGS19 | cg14128040 | 3.98E-04 |
| 81 | BACE2 | cg14523602 | 3.99E-04 |
| 82 | RPH3AL | cg04897931 | 4.07E-04 |
| 83 | MTP18 | cg22395002 | 4.07E-04 |
| 84 | DPP10 | cg26842423 | 4.14E-04 |
| 85 | KIAA1274 | cg04221624 | 4.17E-04 |
| 86 | SCTR | cg22300806 | 4.24E-04 |
| 87 | XKR4 | cg12751432 | 4.43E-04 |
| 88 | SNORD115-15 | cg21751623 | 4.53E-04 |
| 89 | MPRIIP | cg12489964 | 4.53E-04 |
| 90 | PRDM2 | cg21584052 | 4.54E-04 |
| 91 | MAD1L1 | cg20446833 | 4.57E-04 |
| 92 | TOP3B | ch.22.163059F | 4.60E-04 |
| 93 | PRPF39 | cg12773498 | 4.69E-04 |
| 94 | CSF1 | cg14262716 | 4.73E-04 |
| 95 | VSTM2A | cg02866628 | 4.89E-04 |
| 96 | UFM1 | cg04012509 | 4.91E-04 |
| 97 | MCM6 | cg10512742 | 5.00E-04 |
| 98 | TBX1 | cg16332936 | 5.03E-04 |
| 99 | PPT2 | cg12629909 | 5.04E-04 |
| 100 | DDHD1 | ch.14.624270R | 5.05E-04 |
| 101 | FBXL13 | cg05423304 | 5.10E-04 |
| 102 | PRKG1 | cg25997796 | 5.14E-04 |
| 103 | LOC150185 | cg03400811 | 5.16E-04 |
| 104 | HEBP1 | cg03611265 | 5.28E-04 |
| 105 | MIR662 | cg26775123 | 5.35E-04 |

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|-----|----------|------------|----------|
| 106 | ACOX1 | cg19198791 | 5.45E-04 |
| 107 | VPS52 | cg22562590 | 5.51E-04 |
| 108 | XPNPEP3 | cg12638490 | 5.56E-04 |
| 109 | CSDAP1 | cg11706835 | 5.74E-04 |
| 110 | NRP1 | cg25733480 | 5.74E-04 |
| 111 | RPS27 | cg00040380 | 5.76E-04 |
| 112 | UNC80 | cg24938830 | 5.77E-04 |
| 113 | SSTR4 | cg17586860 | 5.87E-04 |
| 114 | NUP93 | cg10586756 | 5.89E-04 |
| 115 | CRCT1 | cg01864564 | 5.92E-04 |
| 116 | SLC16A9 | cg06578117 | 5.94E-04 |
| 117 | CPA2 | cg15942185 | 6.04E-04 |
| 118 | FAM43A | cg02072170 | 6.19E-04 |
| 119 | EIF2AK4 | cg03384128 | 6.23E-04 |
| 120 | PLAGL1 | cg07507918 | 6.29E-04 |
| 121 | DGKD | cg02901644 | 6.42E-04 |
| 122 | GOLSYN | cg08457298 | 6.43E-04 |
| 123 | C5orf60 | cg14851468 | 6.45E-04 |
| 124 | MTMR7 | cg12068916 | 6.59E-04 |
| 125 | PDE4DIP | cg23977954 | 6.59E-04 |
| 126 | PDXK | cg21870229 | 6.62E-04 |
| 127 | SEMA3B | cg22093095 | 6.63E-04 |
| 128 | SLC25A12 | cg04337734 | 6.72E-04 |
| 129 | B4GALT2 | cg23001650 | 6.72E-04 |
| 130 | KLHL24 | cg22757384 | 6.82E-04 |
| 131 | MLL4 | cg15496871 | 6.99E-04 |
| 132 | AGRN | cg07912402 | 7.03E-04 |
| 133 | CEP120 | cg01028844 | 7.03E-04 |
| 134 | HSPA4 | cg13778073 | 7.15E-04 |
| 135 | FRZB | cg14693391 | 7.18E-04 |
| 136 | WDR27 | cg05066503 | 7.29E-04 |
| 137 | DOT1L | cg21880720 | 7.38E-04 |
| 138 | ELL3 | cg16915619 | 7.39E-04 |
| 139 | PRSS42 | cg22550309 | 7.39E-04 |
| 140 | EGFL8 | cg21869704 | 7.51E-04 |
| 141 | CTNNA2 | cg20494682 | 7.55E-04 |
| 142 | TSPYL5 | cg09503853 | 7.55E-04 |
| 143 | BCS1L | cg04970994 | 7.59E-04 |
| 144 | MYH6 | cg05241732 | 7.59E-04 |
| 145 | BAZ2A | cg06607719 | 7.64E-04 |
| 146 | SLC25A22 | cg13749939 | 7.71E-04 |
| 147 | YME1L1 | cg01782320 | 7.77E-04 |
| 148 | C22orf34 | cg20744362 | 7.78E-04 |
| 149 | FARS2 | cg14029759 | 7.84E-04 |

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|-----|----------|------------|----------|
| 150 | BAI1 | cg19721255 | 8.01E-04 |
| 151 | SPIRE2 | cg27072212 | 8.13E-04 |
| 152 | SUSD3 | cg05675356 | 8.29E-04 |
| 153 | FAM155A | cg20343827 | 8.31E-04 |
| 154 | PHRF1 | cg03074210 | 8.32E-04 |
| 155 | ARFGAP3 | cg09861871 | 8.32E-04 |
| 156 | SLC12A7 | cg02350636 | 8.36E-04 |
| 157 | WBSCR28 | cg09117643 | 8.39E-04 |
| 158 | TFB1M | cg27442478 | 8.42E-04 |
| 159 | MYO9A | cg12195460 | 8.47E-04 |
| 160 | ABCC5 | cg10516336 | 8.49E-04 |
| 161 | NMI | cg22664064 | 8.58E-04 |
| 162 | HS6ST3 | cg18748134 | 8.63E-04 |
| 163 | AGAP1 | cg07225241 | 8.66E-04 |
| 164 | SAA2 | cg17310354 | 8.68E-04 |
| 165 | EEF1D | cg16873848 | 8.70E-04 |
| 166 | IKZF5 | cg22262889 | 8.72E-04 |
| 167 | MTNR1B | cg06106126 | 8.72E-04 |
| 168 | CLDN8 | cg22540233 | 8.82E-04 |
| 169 | SLIT3 | cg07819331 | 8.97E-04 |
| 170 | KIAA0408 | cg17215061 | 9.00E-04 |
| 171 | NLRP1 | cg07121721 | 9.05E-04 |
| 172 | KCNQ5 | cg12967218 | 9.16E-04 |
| 173 | CTNND2 | cg19576843 | 9.17E-04 |
| 174 | KLK10 | cg03762081 | 9.18E-04 |
| 175 | SLC9A9 | cg14574489 | 9.24E-04 |
| 176 | MAPK8IP3 | cg02952494 | 9.28E-04 |
| 177 | HCN3 | cg02913804 | 9.28E-04 |
| 178 | HAPLN2 | cg23949901 | 9.32E-04 |
| 179 | DARS | cg11309214 | 9.39E-04 |
| 180 | TLE4 | cg14241138 | 9.40E-04 |
| 181 | MPPED2 | cg23064082 | 9.50E-04 |
| 182 | HCCA2 | cg17139436 | 9.52E-04 |
| 183 | DEFB121 | cg19830128 | 9.54E-04 |
| 184 | ADAMTSL2 | cg00698646 | 9.56E-04 |
| 185 | SRC | cg11210813 | 9.57E-04 |
| 186 | PENK | cg16419235 | 9.63E-04 |
| 187 | FAM155A | cg03333286 | 9.66E-04 |
| 188 | RCSD1 | cg16350225 | 9.78E-04 |
| 189 | ACP1 | cg00431236 | 9.85E-04 |
| 190 | CDH23 | cg22545027 | 9.89E-04 |
| 191 | ARHGAP12 | cg02775617 | 9.89E-04 |
| 192 | CATSPERG | cg14675896 | 9.91E-04 |
| 193 | SLC22A12 | cg09797913 | 9.94E-04 |

Table S2: CpGs of *DPP10* and *SLC16A9* which are differentially methylated in the EtG+ group. Shown are the results obtained from EtG+ vs. controls by independent *t*-tests assuming unequal variances in R (version 3.2.2). The shown regions are obtained from UCSC data base ¹. Bonferroni threshold for *DPP10* $p_{bonf} = .0009$ and for *SLC16A9* $p_{bonf} = .002$, respectively. FDR = false discovery rate.

| Gene | CpG | Region | Methylation | <i>p</i> -Value | FDR |
|-----------------------------|------------|---|-------------|-----------------|------|
| <i>DPP10</i> (51 CpGs) | cg26842423 | Body, Shore | decreased | .0004 | .019 |
| | cg00089091 | Body | decreased | .011 | .238 |
| | cg23260456 | Body | decreased | .022 | .323 |
| <i>SLC16A9</i> (23 CpGs) | cg06578117 | 5'UTR, Enhancer=true | increased | .0006 | .010 |
| | cg02243437 | 1 st Exon, 5'UTR, Enhancer=true | increased | .021 | .175 |

1.2 Figures

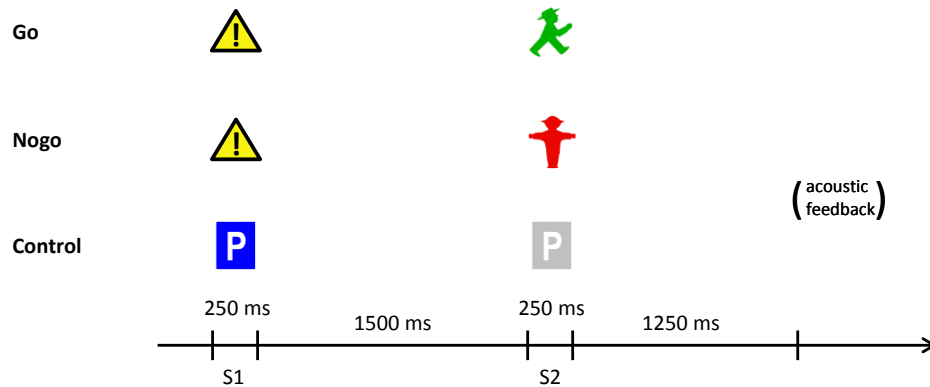


Figure S1: Illustration of the motivational Go/Nogo task (S1-S2 design).

All trial types (control, Go, Nogo) occurred with the same probability. Stimuli were presented for 250 ms. The interstimulus interval between S1 and S2 was 1500 ms, the intertrial interval was set to 3500 ± 500 ms. In two of the four task blocks, monetary incentives were used. Fast responses were rewarded by 10 ct. A fast response was defined individually using the 75th percentile of the reaction times in the previous task block. In turn, the same amount of money was removed if the participant did not react to a Go-stimulus within 1500 ms or when pressing the mouse button in control or Nogo trials. The participants received acoustic feedback for correct or incorrect responses (taken from Heinrich et al. ², p.9.)

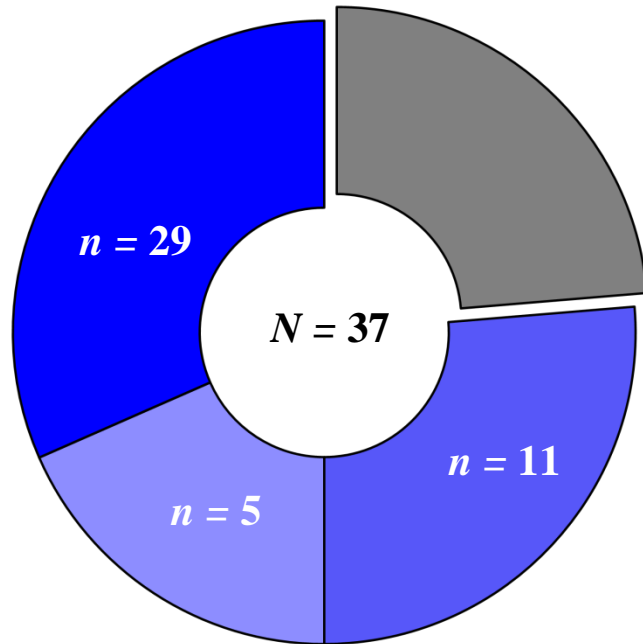


Figure S2: Classification of genes with relevance towards neuronal development according to GO-annotation^{3, 4}. The whole circle represents 37 genes from Table S1 involved in nervous system development (19 %). Among these 37 genes are 29 genes, which are allocated to neurogenesis, as represented by entire blue part. The neurogenesis group is further divided into the subcategories neuronal differentiation ($n = 11$) and axon guidance ($n = 5$).

A

| | Plate | Chip |
|------|-------|------|
| PC1 | | |
| PC2 | | 0.13 |
| PC3 | 0.42 | 0.91 |
| PC4 | | 0.81 |
| PC5 | | 0.45 |
| PC6 | 0.14 | 0.43 |
| PC7 | | 0.13 |
| PC8 | | |
| PC9 | | 0.23 |
| PC10 | | 0.61 |
| PC11 | | |
| PC12 | | 0.13 |
| PC13 | | 0.1 |
| PC14 | | 0.17 |
| PC15 | | 0.22 |
| PC16 | | 0.14 |
| PC17 | | 0.21 |
| PC18 | | 0.11 |
| PC19 | | |
| PC20 | | 0.1 |
| PC21 | | |
| PC22 | | |
| PC23 | | |
| PC24 | | |
| PC25 | | |
| PC26 | | |
| PC27 | | |
| PC28 | | 0.14 |
| PC29 | | |
| PC30 | | |

B

| | Plate | Chip |
|------|-------|------|
| PC1 | | |
| PC2 | | 0.1 |
| PC3 | | |
| PC4 | | 0.1 |
| PC5 | | 0.16 |
| PC6 | | 0.2 |
| PC7 | | 0.15 |
| PC8 | | 0.14 |
| PC9 | | 0.14 |
| PC10 | | 0.13 |
| PC11 | | 0.11 |
| PC12 | | 0.16 |
| PC13 | | |
| PC14 | | 0.13 |
| PC15 | | |
| PC16 | | |
| PC17 | | |
| PC18 | | |
| PC19 | | |
| PC20 | | |
| PC21 | | |
| PC22 | | 0.13 |
| PC23 | | |
| PC24 | | |
| PC25 | | |
| PC26 | | |
| PC27 | | 0.1 |
| PC28 | | |
| PC29 | | |
| PC30 | | |

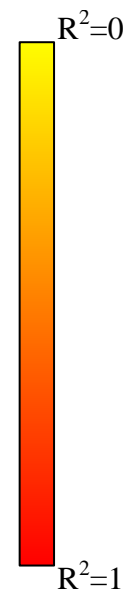


Figure S3. Principle component analysis results indicate technical biases due to plate and chip effects. Shown are R^2 values ≥ 0.1 before (**A**) and after (**B**) correction for 22 control probe PCs.

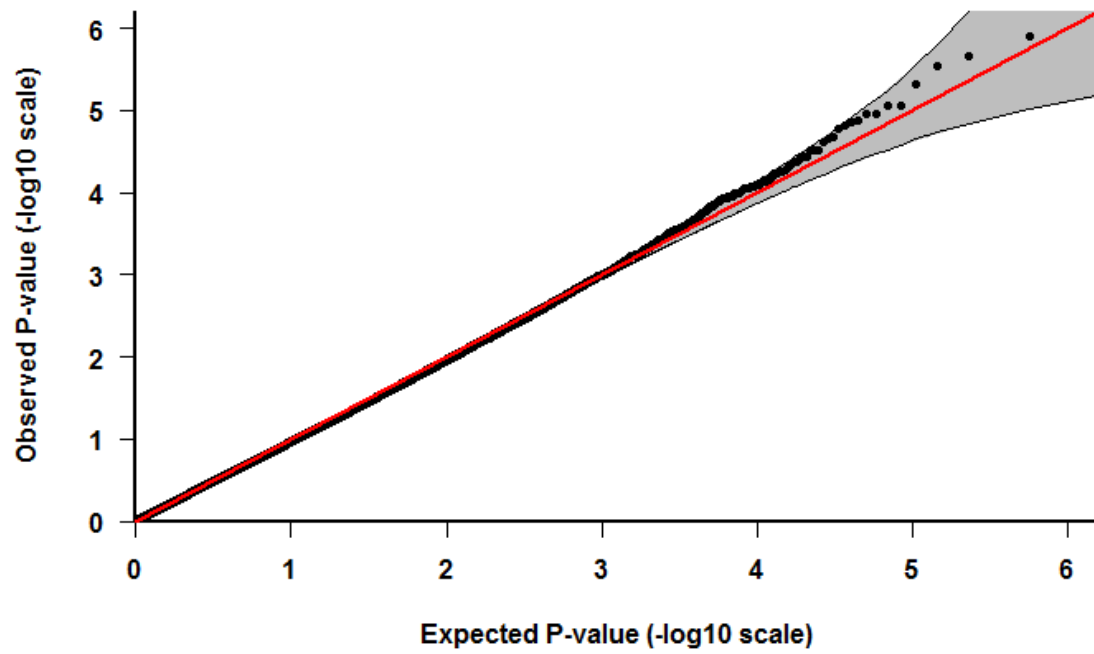


Figure S4: Quantile-quantile (QQ) plot of DNA methylation between EtG positive and control group. The x axis is the expected $-\log_{10} p$ -value, and the y axis is the observed $-\log_{10} p$ -value. $\lambda=0.93$

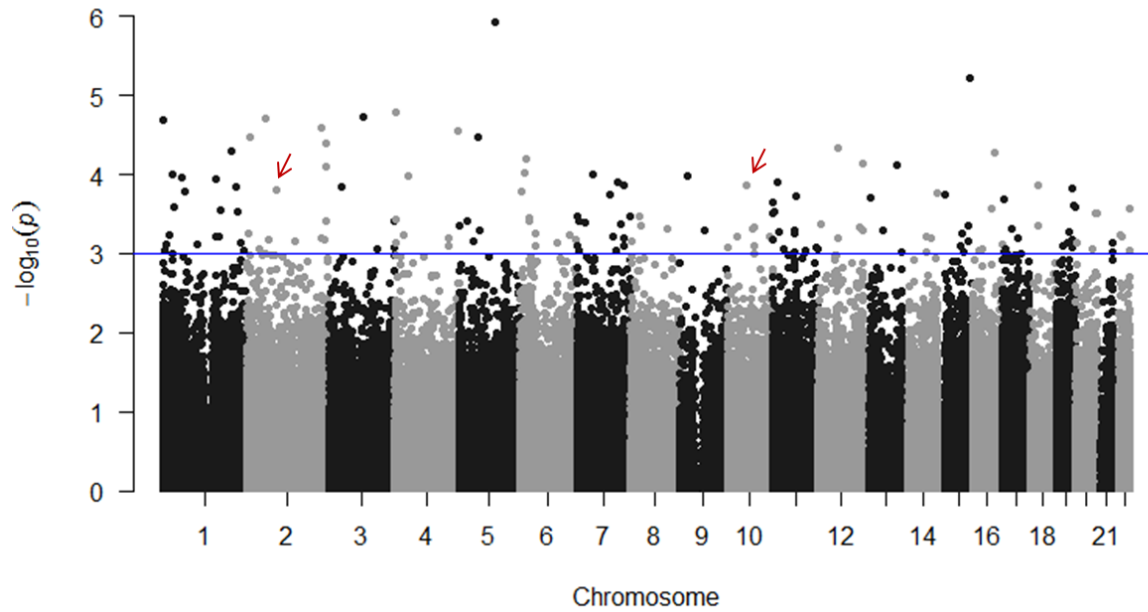


Figure S5: Manhattan plot showing differentially methylated positions plotted according to their chromosomal location with $-\log_{10} p$ -values. The blue line marks the threshold for genes considered in this study (Table S1). The red arrow on chromosome 2 indicates cg26842423 (*DPP10*) and on chromosome10 cg06578117 (*SLC16A9*), respectively.

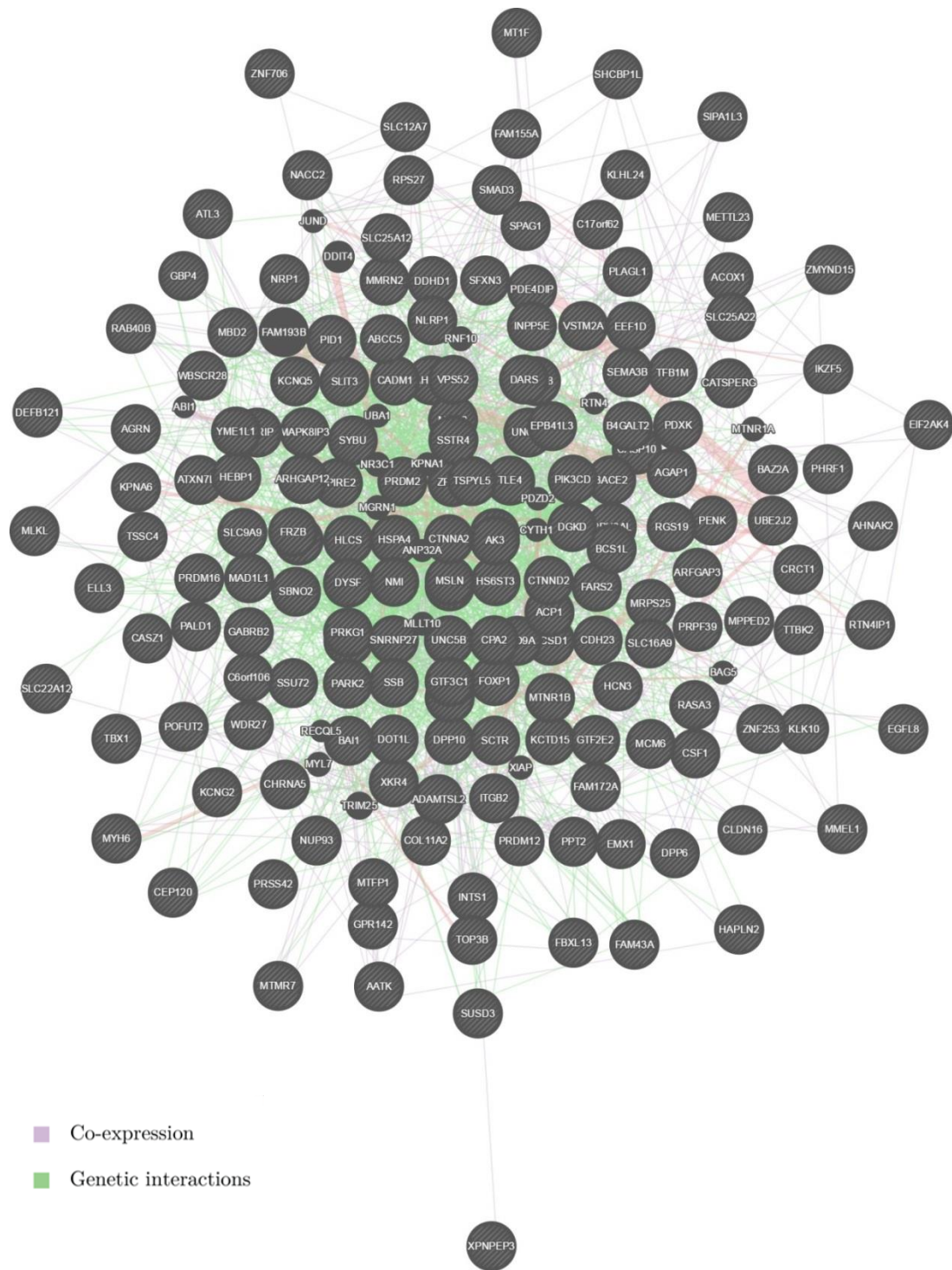


Figure S6: GeneMANIA network for all genes with at least one CpG differentially methylated in EtG+ vs. control children ($p < 10^{-3}$, Table S1).

References

1. Aken BL, Ayling S, Barrell D, Clarke L, Curwen V, Fairley S, *et al.* The Ensembl gene annotation system. *Database (Oxford)* 2016.
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3. Mi H, Huang X, Muruganujan A, Tang H, Mills C, Kang D, *et al.* PANTHER version 11: expanded annotation data from Gene Ontology and Reactome pathways, and data analysis tool enhancements. *Nucleic Acids Res* 2017; 45(D1): D183-D189.
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