

Appendix: Epigenome Wide Association Study Reveals Methylation Loci Associated with Offspring Gestational Diabetes Mellitus Exposure and Maternal Methylome

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Supplementary Table 1: GDM case and control study design with pregnancies group-matched by age and BMI strata

Characteristic	BMI of the mother (kg/m ²)					
	≤ 24.9	≤ 24.9	25.0-29.9	25.0-29.9	≥ 30.0	≥ 30.0
Age of the mother (years)	Control	GDM	Control	GDM	Control	GDM
< 25	9 (1.7 %)	9 (1.7 %)	9 (1.7 %)	9 (1.7 %)	7 (1.3 %)	7 (1.3 %)
25-30	32 (6.0 %)	31 (5.8 %)	21 (3.9 %)	21 (3.9 %)	15 (2.8 %)	20 (3.7 %)
30-35	35 (6.5 %)	35 (6.5 %)	35 (6.5 %)	35 (6.5 %)	10 (1.9 %)	36 (6.7 %)
> 35	34 (6.3 %)	34 (6.3 %)	22 (4.1 %)	33 (6.2 %)	9 (1.7 %)	28 (5.2 %)

An equal number of controls were randomly chosen for each stratum. For older groups of women (> 30 years) with high pre-pregnancy BMI measures (≥ 30), there were insufficient numbers of women who were not affected by GDM. For these groups, all available controls were selected.

Supplementary Table 2: Models used to investigate the two study hypotheses

Model	Question addressed	Equation
1. Shared epigenetic effects (limma)	Are there epigenetic effects (differentially methylated DNA array probes) that are shared between mother and offspring as a result of exposure to GDM?	$M = \gamma \times \text{GDM} + \beta_0 + \beta_1 \times \text{sex} + \beta_2 \times \log(\text{birthweight}) + \beta_3 \times \text{gestationalweek} + \beta_4 \times \text{mumwtgain} + \beta_5 \times \text{Bcell} + \beta_6 \times \text{CD4T} + \beta_7 \times \text{CD8T} + \beta_8 \times \text{Gran} + \beta_9 \times \text{Mono} + \beta_{10} \times \text{NK} + \beta_{11} \times \text{nRBC} + \varepsilon$
2. Generalised model, testing for shared and specific epigenetic effects (Linear mixed model)	<p>Offspring and maternal pooled data analysis:</p> <p>Is there a shared effect of GDM on the methylome of the offspring/mother pairs?</p> <p>Does GDM exposure have a differential impact upon offspring and their mothers? Is there a difference between the methylome of the offspring compared to the mothers?</p>	$M_{\varepsilon} = \gamma_1 \times \text{GDM} + \gamma_2 \times \text{GDM} \times \text{S} + \gamma_3 \times \text{S} + \beta_{0i} + \beta_1 \times \text{sex} + \varepsilon_i,$ <p>with S, the status of the individuals, i.e., offspring or mother</p>
3. Offspring specific epigenetic effects (Linear regression)	<p>Are there differences in methylation between offspring born to mothers with GDM compared to offspring born to mothers without GDM? Specifically, is there a direct association between offspring methylation status and GDM exposure and/or association with GDM exposure modified by the methylome of the mothers?</p>	$M^{\text{Offspring}} = \gamma \times \text{GDM} + \gamma \times \text{GDM} \times M^{\text{Mothers}} + \gamma \times M^{\text{Mothers}} + \beta_0 + \beta_1 \times \text{sex} + \beta_2 \times \log(\text{birthweight}) + \beta_3 \times \text{gestationalweek} + \beta_4 \times \text{Bcell} + \beta_5 \times \text{CD4T} + \beta_6 \times \text{CD8T} + \beta_7 \times \text{Mono} + \beta_8 \times \text{NK} + \varepsilon,$ <p>with the covariate M^{Mothers} above being the residuals (ε) from the following linear model:</p> $M^{\text{Mothers}} = \beta_0 + \beta_1 \times \text{age} + \beta_2 \times \text{BMI} + \beta_3 \times \text{mumwtgain} + \beta_4 \times \text{Bcell} + \beta_5 \times \text{CD4T} + \beta_6 \times \text{CD8T} + \beta_7 \times \text{Mono} + \beta_8 \times \text{NK} + \beta_9 \times \text{Neu} + \varepsilon.$ <p>Offspring/mother M pairs were retained for those following a distribution of $Q_{25}(M) - 3 \times \text{IQR}(M) \leq M \leq Q_{75}(M) + 3 \times \text{IQR}(M)$</p>

Supplementary Table 3: GDM EWAS results for offspring with $p < 0.05$ (Full Table in EXCEL)

Supplementary Table 4: GDM EWAS results for mothers with $p < 0.05$ (Full table in EXCEL)

Supplementary Table 5: Details of methylation changes associated with gestational GDM exposure

Term ¹	CpG ID	Estimate (M) ²	Estimate (β) ²	Average β_{value}	Number of Mother/Offspring Pair ³	P-Value ⁴	FDR	Relation to CpG Island	Gene	Location in Gene
GDM	cg22790973	-1.56 ± 0.271	-0.00251 ± 0.00170	1.03 %	401	1.90×10^{-8}	1.38×10^{-2}	Island	<i>TFCP2</i>	TSS1500
GDM \times Mother	cg22790973	0.528 ± 0.0826	0.0579 ± 0.140	1.03 %	401	4.96×10^{-10}	3.59×10^{-4}	Island	<i>TFCP2</i>	TSS1500
GDM \times Mother	cg03456133	0.569 ± 0.0949	0.775 ± 0.0762	96.48 %	498	4.02×10^{-9}	1.46×10^{-3}	Open Sea	—	—
GDM \times Mother	cg19107264	0.574 ± 0.102	0.486 ± 0.0877	96.64 %	502	3.05×10^{-8}	7.37×10^{-3}	South Shelf	<i>DLGAP2</i>	Body
GDM \times Mother	cg23355087	-0.473 ± 0.0851	-0.602 ± 0.0712	95.66 %	484	4.55×10^{-8}	8.24×10^{-3}	Open Sea	—	—
GDM \times Mother	cg20002843	-0.295 ± 0.0537	-0.347 ± 0.0616	47.37 %	503	6.68×10^{-8}	9.68×10^{-3}	Open Sea	<i>LOC127841</i>	Body
GDM \times Mother	cg24440941	-0.496 ± 0.0917	-0.482 ± 0.168	1.48 %	492	1.03×10^{-7}	1.24×10^{-2}	North Shore	<i>H3C6</i>	TSS1500
GDM \times Mother	cg17065901	0.600 ± 0.113	0.751 ± 0.0722	98.25 %	498	1.70×10^{-7}	1.76×10^{-2}	Open Sea	<i>FAM13A</i>	Body
GDM \times Mother	cg11493553	-0.509 ± 0.0975	-0.663 ± 0.0944	97.07 %	478	2.66×10^{-7}	2.41×10^{-2}	Open Sea	<i>UBE3C</i>	Body

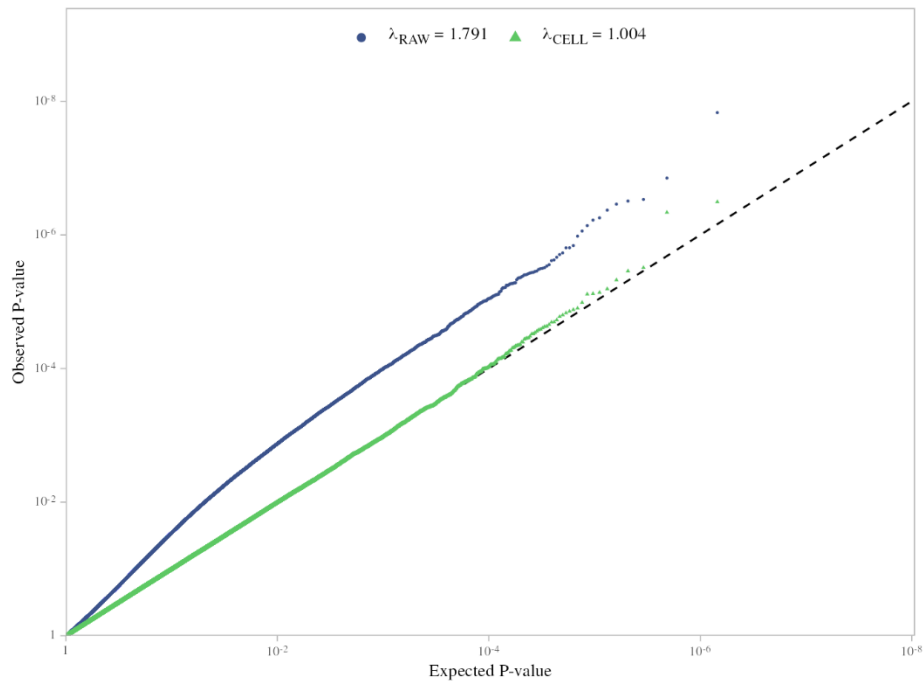
¹ Variable term or interaction term from the linear regression, where "Mothers" is the methylation of the mothers as M_{value}

² Linear regression coefficient plus/minus standard error on the M_{value} or β_{value} scale.

³ Number of Mother/Offspring pair after outliers exclusion, with M_{value} greater than 3 times the interquartile range below the 25th percentile and lower than 3 times the interquartile range above the 75th percentile.

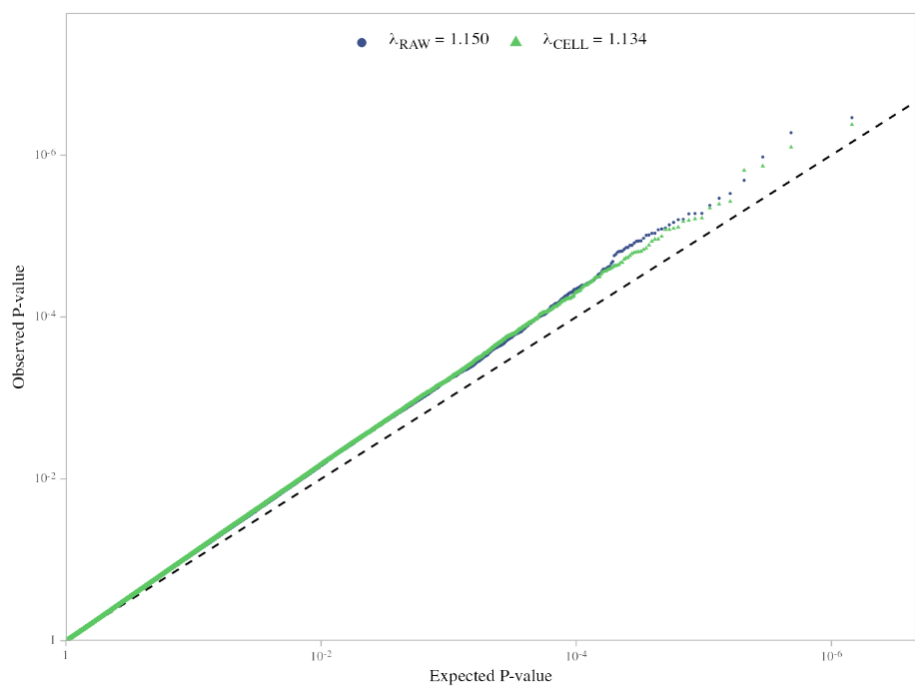
⁴ Linear regression p-value computed on the M_{value} scale.

Supplementary Figure 1: Probability-Probability plots for offspring EWAS



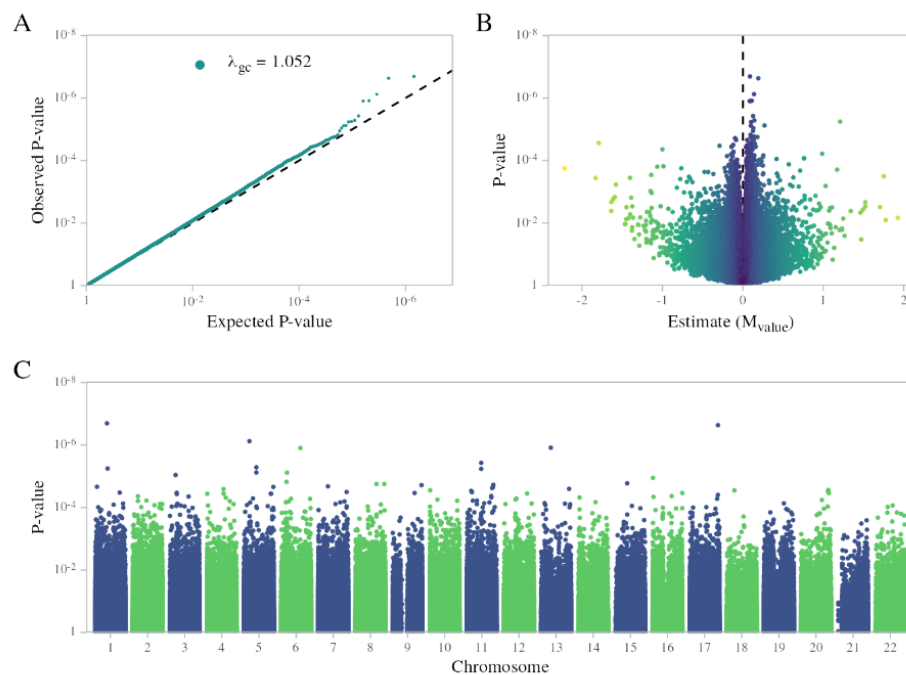
Probability-Probability plots for offspring EWAS. Linear regression between offspring methylation and maternal GDM exposure, adjusted for sex, gestational week, birthweight with or without cell composition.

Supplementary Figure 2: Probability-Probability plots for mothers EWAS



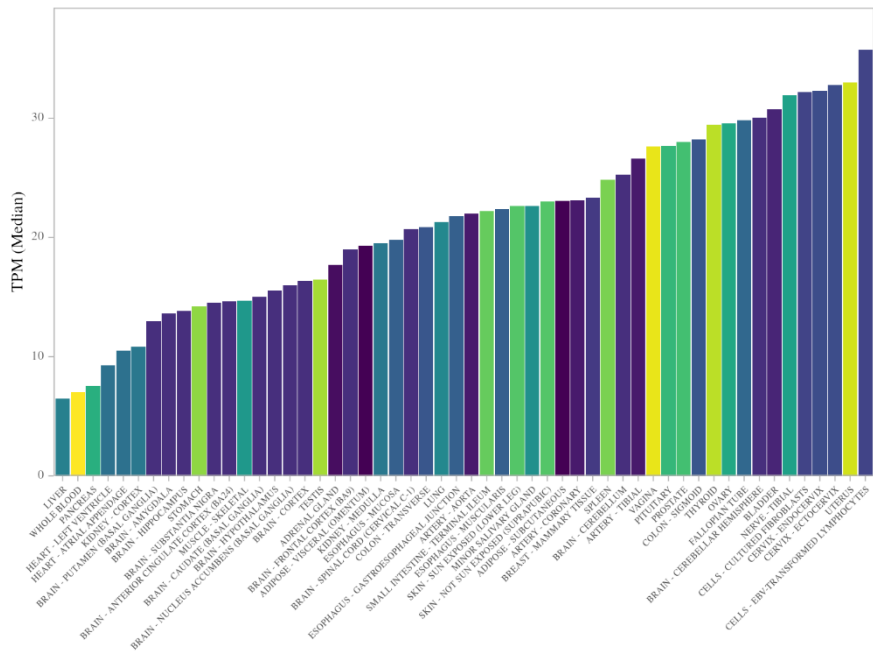
Probability-Probability plots for mothers EWAS. Linear regression between maternal methylation and maternal GDM exposure, adjusted for age and BMI with or without cell composition.

Supplementary Figure 3: EWAS results for fixed effect GDM of the linear mixed model using both offspring and mothers' methylome as response



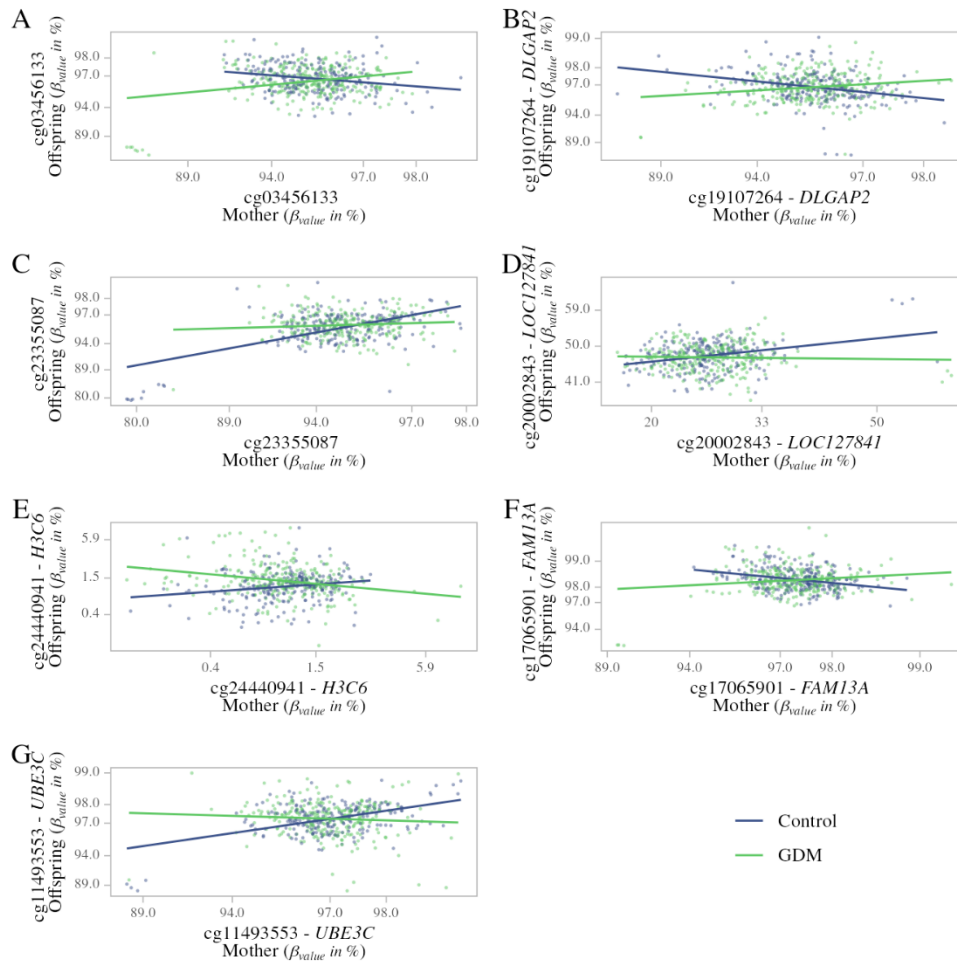
EWAS results for fixed effect GDM of the linear mixed model using both offspring and mothers' methylome as response. A) Probability-Probability plot of the GDM exposure fixed effect on the methylation of both offspring and mothers, with the black line indicating the expected distribution. B) A volcano plot and C) Manhattan plot, showing the genome-wide results for all the CpGs.

Supplementary Figure 4: Ubiquitous expression of *TFCP2* in different tissues using GTEx



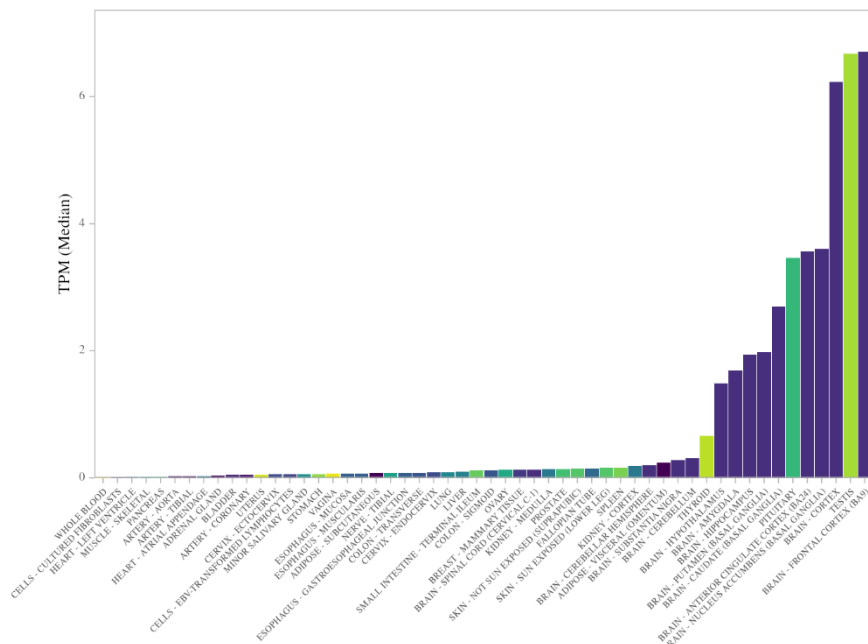
Ubiquitous expression of *TFCP2* in different tissues using GTEx. The global expression pattern of *TFCP2* shows a ubiquitous expression pattern.

Supplementary Figure 5: CpGs with a GDM exposure interaction with maternal methylation on the methylation of the offspring



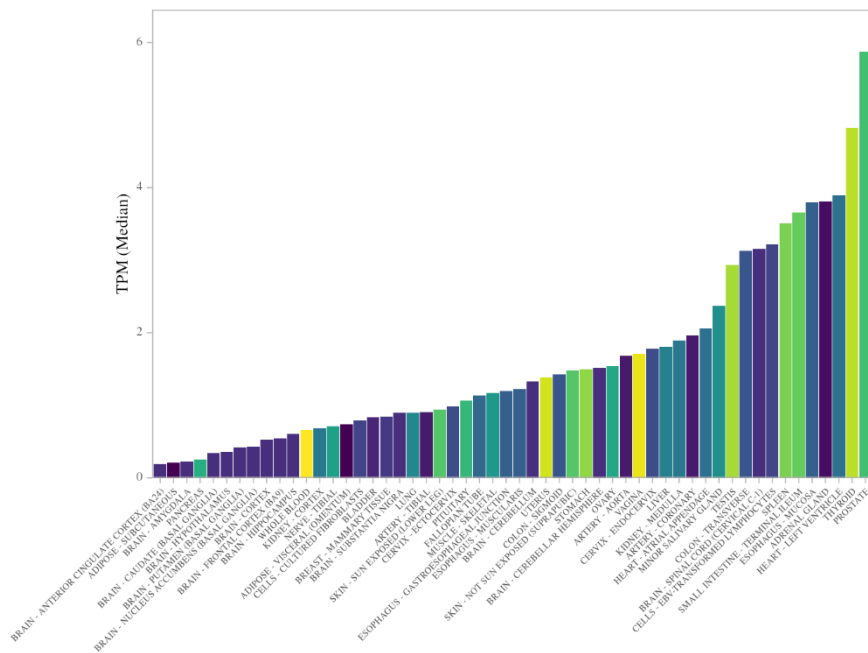
Seven CpGs with a significant GDM exposure interaction with mother methylation on the methylation of the offspring. Scatterplots showing the differences in methylation between offspring and mother according to the maternal GDM exposure in the A) cg03456133 located within an intergenic region, B) cg19107264 in *DLGAP2* gene, C) cg17065901 in *FAM13A* gene, D) cg2335508 located within an intergenic region and E) cg24440941 in *H3C6*, F) cg20002843 located in *LOC127841* gene body and G) cg11493553 in *UBE3C*.

Supplementary Figure 6: Expression of *DLGAP2* in different tissues using GTEx



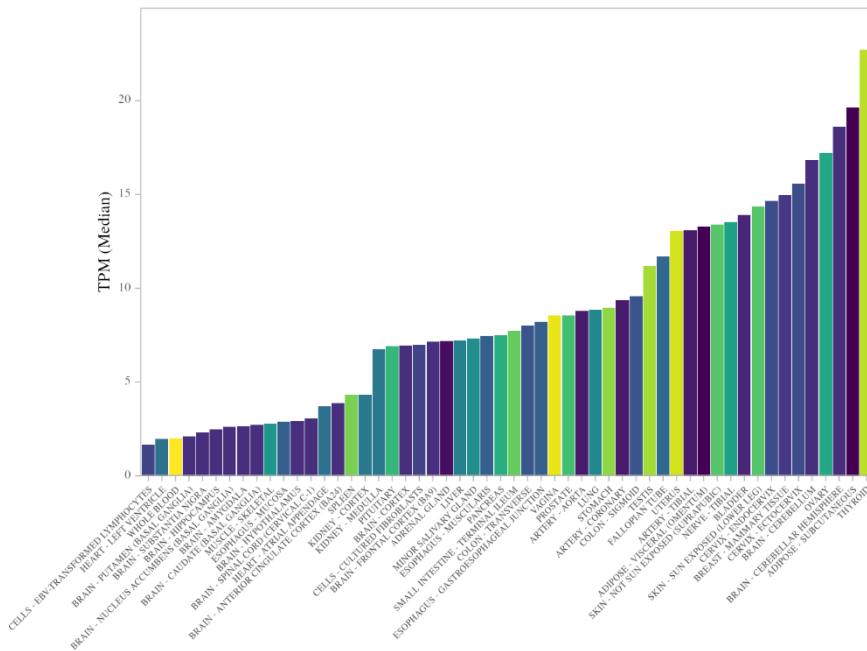
Expression of *DLGAP2* in different tissues using GTEx. The global expression pattern of *DLGAP2* from GTEx shows an expression pattern in brain.

Supplementary Figure 7: Expression of *H3C6* in different tissues using GTEx



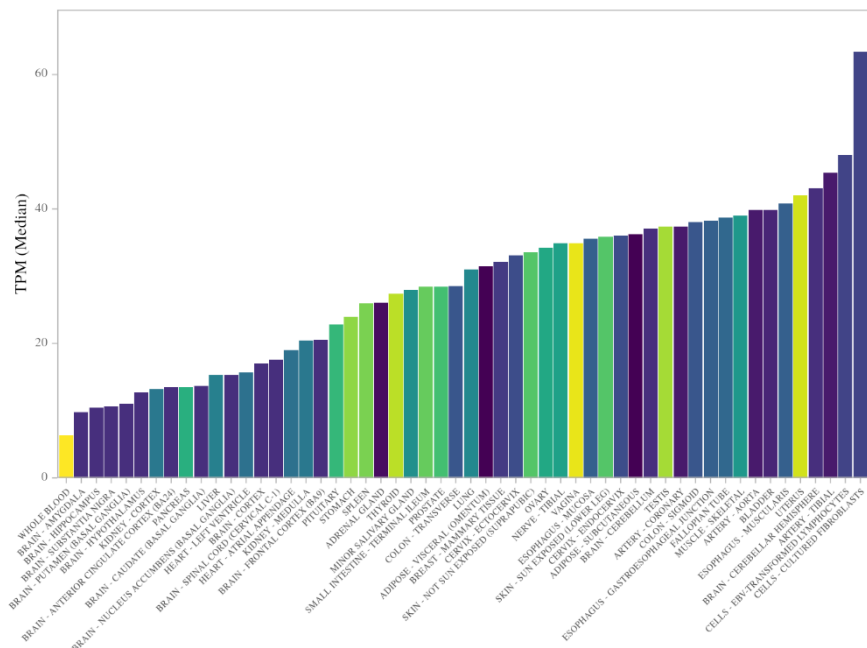
Expression of *H3C6* in different tissues using GTEx. The global expression pattern of *H3C6* from GTEx shows an expression pattern in brain.

Supplementary Figure 8: Expression of *FAM13A* in different tissues using GTEx



Expression of *FAM13A* in different tissues using GTEx. The global expression pattern of *FAM13A* from GTEx shows an expression pattern in brain.

Supplementary Figure 9: Expression of *UBE3C* in different tissues using GTEx



Expression of *UBE3C* in different tissues using GTEx. The global expression pattern of *UBE3C* from GTEx shows an expression pattern in brain.