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# **Supplemental Material**

### Residential Proximity to Major Roadways at Birth, DNA Methylation at Birth and Midchildhood, and Childhood Cognitive Test Scores: Project Viva (Massachusetts, USA)

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# Additional File - Excel Document

**Table S1.** Summary statistics of FDR-significant CpG sites (for the association between residential proximity to roadways at birth and cord blood DNA methylation) in cord blood (N=482) and in peripheral blood (N=457) in Project Viva. Summary statistics are present on the beta-value scale (0%-100% methylation).

			Percentile		
Cord blood	Mean	SD	10 <sup>th</sup>	50 <sup>th</sup>	90 <sup>th</sup>
cg05654765	27.1%	6.5%	19.0%	26.5%	36.6%
cg14099457	35.4%	7.3%	26.8%	34.9%	45.4%
cg03732535	7.0%	2.2%	4.5%	6.8%	9.8%
cg02954987	42.0%	10.1%	29.6%	41.5%	56.0%
Peripheral blood	Mean	SD	10 <sup>th</sup>	50 <sup>th</sup>	90 <sup>th</sup>
cg05654765	39.4%	7.4%	30.0%	39.0%	49.8%
cg14099457	51.2%	9.3%	40.2%	50.8%	64.4%
cg03732535	21.4%	5.6%	14.1%	21.2%	29.2%
cg02954987	63.3%	11.0%	49.4%	63.5%	77.8%

**Table S2.** Differential methylation (%) (95% CI) in cord blood DNA associated with a 50% reduction in prenatal residential proximity to major roadways, stratified by sex and race/ethnicity.

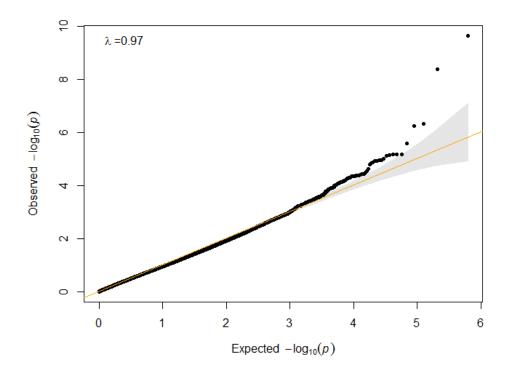
	All <sup>a</sup>		White <sup>b</sup>		Non-white <sup>c</sup>		
CpG	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI	<b>p</b> interaction
cg05654765	0.82%	0.54%; 1.10%	0.77%	0.44; 1.12	0.97%	0.40%; 1.53%	0.465
cg14099457	0.88%	0.56%; 1.19%	0.85%	0.48; 1.23	0.92%	0.32%; 1.55%	0.589
cg03732535	0.19%	0.11%; 0.28%	0.15%	0.05%; 0.26%	0.31%	0.16%; 0.47%	0.078
cg02954987	1.08%	0.65%; 1.51%	1.10%	0.59; 1.62	0.98%	0.13%; 1.84%	0.955

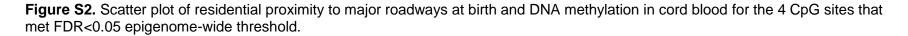
	Male <sup>d</sup>		F		
CpG	Estimate	95% CI	Estimate	95% CI	<b>p</b> interaction
cg05654765	0.68%	0.29%; 1.07%	1.00%	0.56%; 1.41%	0.316
cg14099457	0.79%	0.36%; 1.22%	1.01%	0.53%; 1.50%	0.516
cg03732535	0.15%	0.03%; 0.27%	0.28%	0.15%;0.42%	0.215
cg02954987	1.05%	0.46%; 1.65	1.22%	0.57%; 1.87%	0.715

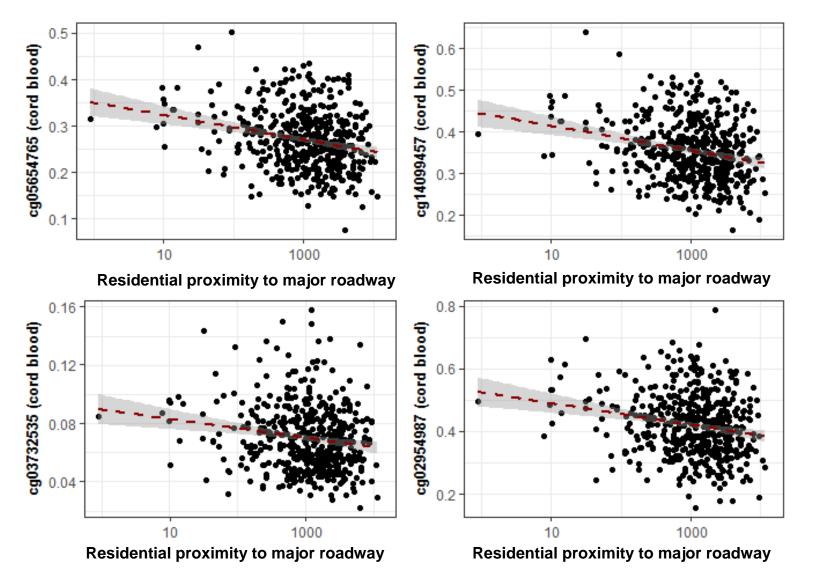
Model adjusted for maternal [age at enrollment (continuous), pre-pregnancy BMI (continuous), race (white / black / others), smoking status (smoking during pregnancy / former / never), education level (college or graduate / not college or graduate)], neighborhood [median household income (continuous)], children [child's sex (female / male), gestational age (continuous), season (DOB)], and estimated cell proportions (percentage of monocyte, percentage of CD8T cell, percentage of CD4T cell, percentage of NK cell, percentage of B cell, percentage of nucleated red blood cell).

<sup>a</sup> N=482; <sup>b</sup> N=342; <sup>c</sup> N=140; <sup>d</sup> N=230; <sup>e</sup> N=252.

**Figure S1.** Q-Q plot of the association between residential proximity to roadway at birth and cord blood epigenome-wide DNA methylation (x-axis is the expected –log10(p-value), y-axis is the observed –log10(p-value), lambda = 0.97).







**Figure S3.** Scatter plot of DNA methylation in cord blood of newborns and peripheral blood at mid-childhood for the 4 CpG sites that met FDR<0.05 epigenome-wide threshold.

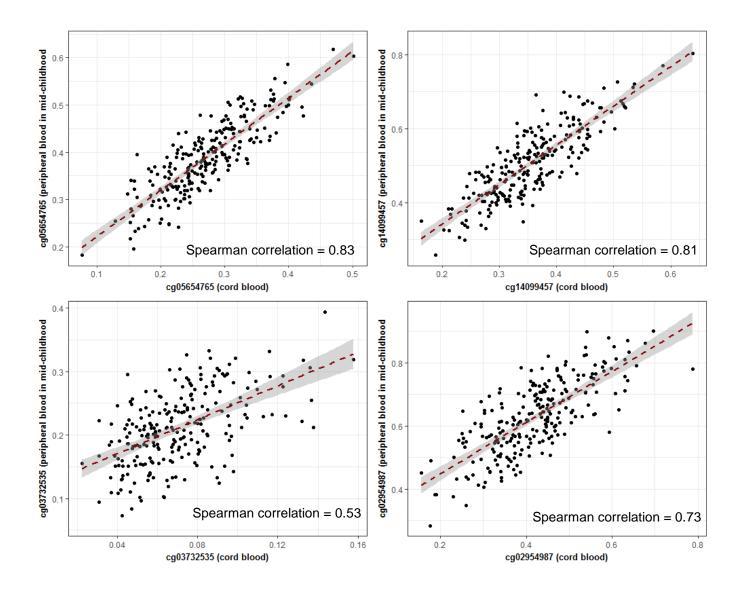
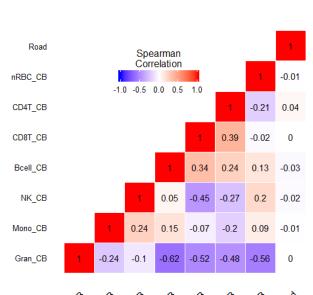


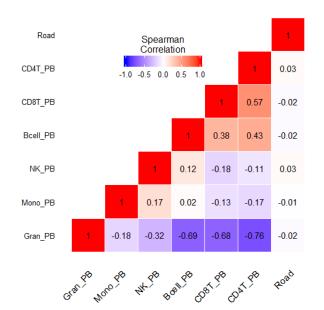
Figure S4. Correlation between residential proximity to major roadways at birth and cell proportions (a) in cord blood and (b) in peripheral blood at mid-childhood.

(b)



(a)





cg14099457 cg05654765 0.6 0.5 0.5 0.4 Methylation 0.2 0.2 DNA Methylation 0.2 0.1 0.1 PFC EC stg CER Blood PFC EC STG CER Blood r = 0.715 p value = 8.77e-13 r = 0.654 p value = 6.05e-10 r = 0.655 p value = 2.46e-10 r = 0.727 p value = 6.93e-13 n = 74 n = 74 n = 71 n = 71 0.5 0.6 0.6-0.5 0.5 0.5-0.4 0.4 0.4-2 ( ස 0.4 입 0.3 입 0.3· Ы 0.3 0.3-0.2 0.2 0.2 0.2-0.1 0.1 0.1 0.1 0.2 0.1 0.2 0.5 0.1 0.2 0.5 0.1 0.3 0.4 0.5 0.6 0.1 0.2 0.3 0.4 0.5 0.6 0.3 0.4 0.3 0.4 Blood Blood Blood Blood r = 0.684 p value = 1.33e-11 r = 0.102 p value = 0.4 r = 0.589 p value = 2.66e-08 r = 0.057 p value = 0.637 n = 75 n = 71 n = 75 n = 71 0.5 0.5 0.6-0.6-0.5 0.5-0.4 0.4 0.4 0.4 ( 0 S 0.3⊐ | ∰ 0.3→ 0 0 0.3 띥 0.3 0.2 0.2 0.2-0.2-

0.1

0.2

0.3

Blood

0.4

0.5

0.6

0.1

0.1

0.1

100 10 300

0.3

Blood

0.4

0.5

0.6

0.2

0.1

0.1

0.2

0.4

0.3

Blood

0.5

0.1

0.1

0.2

0.4

0.3

Blood

0.5

**Figure S5.** A comparison between blood and post-mortal brain tissues (prefrontal cortex, entorhinal cortex, superior temporal gyrus and cerebellum) for the FDR significant CpGs we identified.

cg02954987 cg03732535 0.4 0.6 DNA Methylation DNA Methylation 0.3 0.2 0.1 0.1 PFC EC STG CER Blood PFC EC STG CER Blood r = 0.683 p value = 2.02e-11 r = 0.247 p value = 0.0338 r = 0.618 p value = 9.14e-09 r = 0.164 p value = 0.171 n = 74 n = 71 n = 74 n = 71 0.4 0.4 0.6-0.6-0.5 0.5 0.3 0.3 ე 0.4∙ Ⴞ PFC 0.4 Ы В 0.3 0.3 0.2 0.2-0.2 0.2-0.1 0.1 0.1 0.1 0.1 0.2 0.3 0.4 0.5 0.6 0.1 0.2 0.3 0.4 0.5 0.6 0.1 0.2 0.3 0.4 0.1 0.2 0.3 0.4 Blood Blood Blood Blood r = 0.658 p value = 1.42e-10 r = 0.173 p value = 0.148 r = 0.2 p value = 0.0846 r = -0.0298 p value = 0.805 n = 75 n = 71 n = 75 n = 71 0.4 0.4 0.6-0.6 0.5 0.5 0.3 0.3 ე 0.4 ყე 띥 <sup>0.4·</sup> STG СË 0.3 0.3 0.2 0.2-0.2 0.2-0.1 0.1 0.1 0.1 1 12.00 0.2 0.2 0.1 0.3 0.1 0.3 0.4 0.5 0.6 0.1 0.3 0.4 0.5 0.6 0.1 0.2 0.3 0.4 0.2 0.4 Blood Blood Blood Blood

Figure S5 continued. A comparison between blood and post-mortal brain tissues (prefrontal cortex, entorhinal cortex, superior temporal gyrus and cerebellum) for the FDR significant CpGs we identified.