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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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**	**10th**	**50th**	**90th**
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**	**10th**	**50th**	**90th**
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.

CpG	All[a]		White[b]		Non-white[c]		$p_{interaction}$
	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI	
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

| CpG	Male[d]		Female[e]		$p_{interaction}$
	Estimate	95% CI	Estimate	95% CI	
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).

[a] N=482; [b] N=342; [c] N=140; [d] N=230; [e] 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).
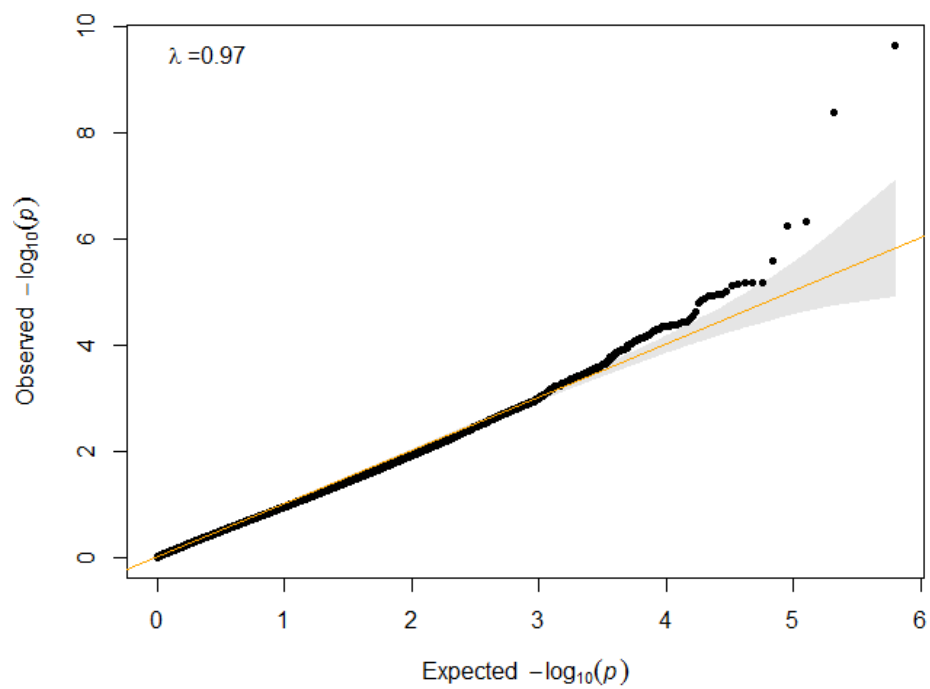
Figure S2. Scatter plot of residential proximity to major roadways at birth and DNA methylation in cord blood for the 4 CpG sites that met FDR<0.05 epigenome-wide threshold.
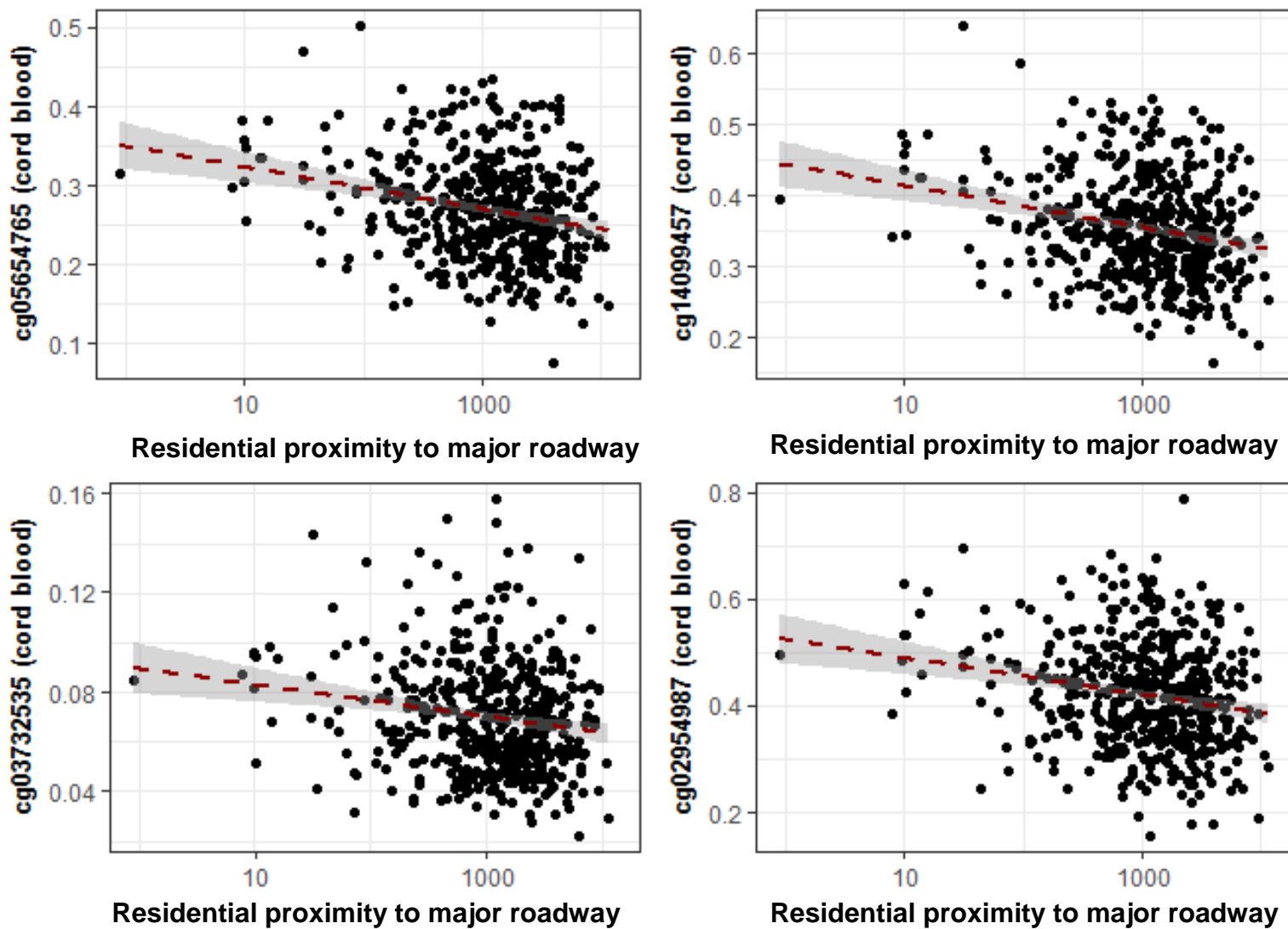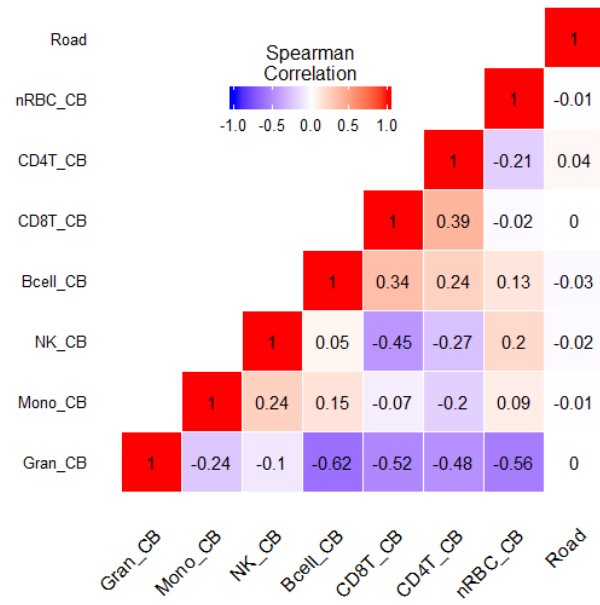
Figure S3. Scatter plot of DNA methylation in cord blood of newborns and peripheral blood at mid-childhood for the 4 CpG sites that met FDR<0.05 epigenome-wide threshold.
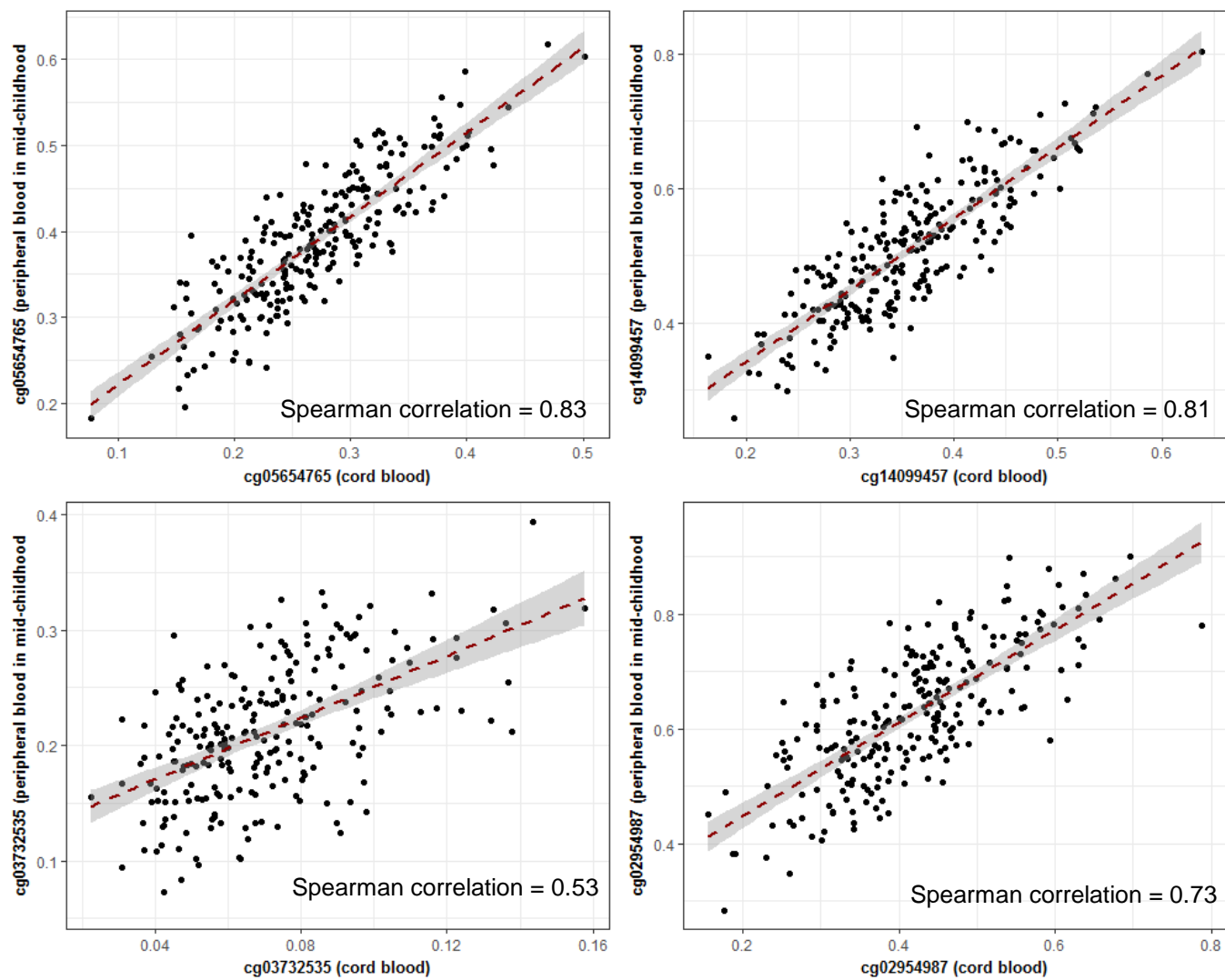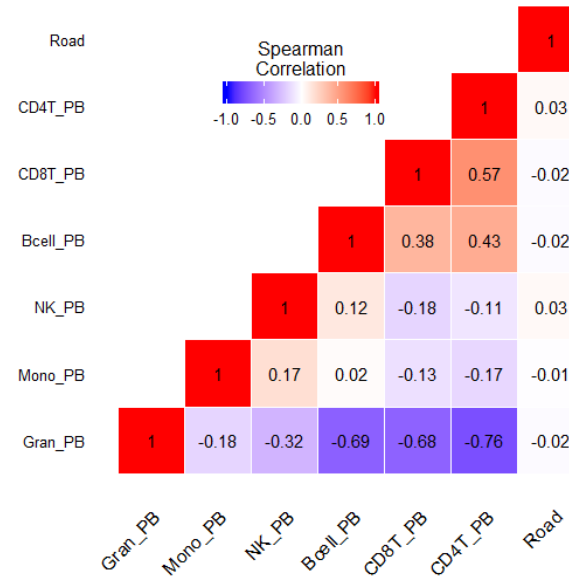
Figure S4. Correlation between residential proximity to major roadways at birth and cell proportions (a) in cord blood and (b) in peripheral blood at mid-childhood.

(a)

(b)

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.

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.