

Placental promoter methylation of DNA repair genes and prenatal exposure to particulate air pollution: an ENVIRONAGE cohort study.

Supplementary material

NEVEN, Kristof; SAENEN, Nelly; Tarantini, Letizia; JANSSEN, Bram; Lefebvre, Wouter; Vanpoucke, Charlotte; Bollati, Valentina & NAWROT, Tim (2018) Placental promoter methylation of DNA repair genes and prenatal exposure to particulate air pollution: an ENVIRONAGE cohort study.. In: Lancet Planetary Health, 2(4), p. e174-e183.

DOI: 10.1016/S2542-5196(18)30049-4

Handle: <http://hdl.handle.net/1942/25905>

# THE LANCET Planetary Health

## Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed.  
We post it as supplied by the authors.

Supplement to: Neven K Y, Saenen N D, Tarantini L, et al. Placental promoter methylation of DNA repair genes and prenatal exposure to particulate air pollution: an ENVIRONAGE cohort study. *Lancet Planet Health* 2018; 2: e174–83.

## **APPENDIX**

**Supplemental Table S1: Information regarding gene assembly, amount of CpG sites and the primer sequences.**

**Supplemental Table S2: Trimester specific exposure windows.**

**Supplemental Table S3: Clinical and socio-demographic characteristics of the participants in the total group, in the ENVIRONAGE birth cohort, and reference values from Flanders.**

**Supplemental Table S4: DNA methylation information.**

**Supplemental Table S5: Association between mutation marker, and DNA repair genes in association with PM<sub>10</sub>, PM<sub>2.5</sub>, black carbon (BC), and NO<sub>2</sub> exposure during the entire pregnancy.**

**Supplemental Figure S1: Maps of the province of Limburg (Flanders, Belgium).**

**Supplemental Figure S2: Sensitivity analysis.**

**Supplemental Table S1. Information regarding gene assembly, amount of CpG sites and the primer sequences.** For all genes, UCSC assembly hg38 was used, with the exception of *PARP1*, for which hg19 was consulted. For all assays, the amount of CpG sites that were interrogated are shown, except for *Alu* where the amount of mutation spots are presented. Sequences of the primers from left to right are from 5' to 3'.

Gene	Chromosome location	CpG sites	Forward (F), Reverse (R) and Sequencing (Seq) primers
<i>APEX1</i>	chr14:20455191-20457772	3	F: GGTTAGGAGGAGTTAGGTTGTTAT R: <i>BIOTIN</i> -CCAACCAATTAAAAATCTTAATTAA Seq: GGTTAGGAGGAGTTAGGTTGTTAT
<i>PARP1</i>	chr1:226590392-226597000	7	F: TGATAGATTGTTGATGTTTGGT R: <i>BIOTIN</i> -AACTACTAACTCAACCCAAACCC Seq: TGATAGATTGTTGATGTTTGGT
<i>OGG1</i>	chr3:9749944-9757407	3	F: GGGATTATATTTTAGGAAAGT R: <i>BIOTIN</i> -ATACTATTTAACAACCTTCCCTATT Seq: GGGATTATATTTTAGGAAAGT
<i>ERCC1</i>	chr19:45407333-45424365	4	F: TTTGGTTTTGTTTATTTTATAGATT R: <i>BIOTIN</i> -TCTACAATCTTCCCTTAAAACTCC Seq: TTTGGTTTTGTTTATTTTATAGATT
<i>ERCC4</i>	chr16:13917894-13922722	5	F: GATAGTTGGTGTGGAATTGTT R: <i>BIOTIN</i> -CCCCTACCATCCTTCTCTATATCA Seq: TTGGTGTGGTGTGTTAAT
<i>p53</i>	chr17:7531143-7531743	5	F: <i>BIOTIN</i> -TTAGGAGTTTATTTAATTTAGGGAAG R: TATCCAACCTTTATACCAAAAACCTC Seq: TCCAACCACTTTATACCAAAAACCTC
<i>DAPK1</i>	chr9:90112116-90113558	3	F: AGGTAGGGATTTAAAAAATTTGT R: <i>BIOTIN</i> -CCCACCTATAACACATTACTAAAC Seq: TGGGATTTTAGTATATATTT
Assay	Chromosome location	Mutation sites	Primers: Forward (F), Reverse (R) and Sequencing (Seq)
<i>Alu</i>	-	3	F: <i>BIOTIN</i> -TTTTTATTAATAATAATAATAATT R: CCCAACTAAATACAATAA Seq: AATAACTAAAATTACAAAC

**Supplemental Table S2. Trimester specific exposure windows.** All models were adjusted for the following covariates: newborns' sex, ethnicity, parity, maternal age, maternal education, smoking habits, pre-pregnancy BMI, gestational age, season at delivery, and batch effect. In order to obtain a relevant exposure change, the effect estimates ( $\beta$ ) of the mutation marker and genes were presented as a relative percentage change in methylation level for an interquartile range (IQR) increment in air pollution exposure during the specific exposure windows. PM<sub>2.5</sub>: 8.14  $\mu\text{g}/\text{m}^3$  for the first trimester, 7.36  $\mu\text{g}/\text{m}^3$  for the second trimester, 8.19  $\mu\text{g}/\text{m}^3$  for the third trimester, and 3.84  $\mu\text{g}/\text{m}^3$  for the entire pregnancy. Black carbon (BC): 0.54  $\mu\text{g}/\text{m}^3$  for the first trimester, 0.51  $\mu\text{g}/\text{m}^3$  for the second trimester, 0.52  $\mu\text{g}/\text{m}^3$  for the third trimester, and 0.36  $\mu\text{g}/\text{m}^3$  for the entire pregnancy.

	<i>ALU</i>		<i>APEXI</i>		<i>OGGI</i>		<i>ERCC4</i>		<i>p53</i>		<i>DAPKI</i>	
	$\beta$ (95% CI)	p-value	$\beta$ (95% CI)	p-value	$\beta$ (95% CI)	p-value	$\beta$ (95% CI)	p-value	$\beta$ (95% CI)	p-value	$\beta$ (95% CI)	p-value
<b>Trimester 1 PM<sub>2.5</sub> exposure</b>	1.0 (-2.3 to 4.3)	0.57	5.6 (-3.4 to 14.7)	0.22	2.5 (-9.1 to 14.1)	0.67	27.9 (10.4 to 45.4)	0.0022	11.4 (3.5 to 19.4)	0.01	-2.7 (-12.8 to 7.4)	0.60
<b>Trimester 2 PM<sub>2.5</sub> exposure</b>	2.3 (-0.7 to 5.3)	0.13	4.2 (-4.2 to 12.5)	0.33	8.5 (-2.3 to 19.3)	0.12	17.2 (1.2 to 33.2)	0.036	4.3 (-3.1 to 11.6)	0.26	-12.8 (-20.9 to -4.7)	0.0019
<b>Trimester 3 PM<sub>2.5</sub> exposure</b>	2.4 (-0.8 to 5.7)	0.15	9.0 (0.5 to 17.5)	0.0033	16.5 (5.5 to 27.6)	0.0032	27.0 (10.6 to 43.3)	0.0009	-0.4 (-7.9 to 7.1)	0.92	-1.6 (-12.6 to 9.3)	0.77
<b>Entire Pregnancy PM<sub>2.5</sub> exposure</b>	3.0 (0.2 to 5.7)	0.035	9.0 (2.3 to 15.7)	0.0047	13.9 (5.1 to 22.6)	0.0049	16.3 (5.4 to 27.2)	0.003	10.6 (4.5 to 16.7)	0.0012	-12.9 (-22.3 to -3.5)	0.0077
<b>Trimester 1 BC exposure</b>	1.2 (-2.1 to 4.5)	0.50	5.2 (-4.3 to 14.7)	0.44	-6.0 (-15.8 to 3.9)	0.23	42.8 (13.0 to 66.8)	0.0039	1.5 (-29.7 to 47.3)	0.65	-1.7 (-12.3 to 7.8)	0.66
<b>Trimester 2 BC exposure</b>	1.9 (0.0 to 3.8)	0.049	6.5 (-2.7 to 15.7)	0.38	7.0 (-4.7 to 18.6)	0.24	10.9 (1.0 to 20.8)	0.042	1.2 (-43.3 to 45.7)	0.96	-12.0 (-23.1 to -0.9)	0.014
<b>Trimester 3 BC exposure</b>	2.6 (1.5 to 3.7)	<0.0001	9.3 (2.1 to 16.5)	0.0078	-14.8 (-14.8 to 5.2)	0.24	2.6 (-7.3 to 12.4)	0.61	8.9 (-28.8 to 46.7)	0.64	-1.9 (-9.8 to 6.0)	0.55
<b>Entire Pregnancy BC exposure</b>	2.0 (1.4 to 2.5)	0.0013	9.2 (4.1 to 14.3)	0.0018	-2.0 (-8.9 to 4.9)	0.27	27.6 (17.6 to 37.6)	<0.0001	2.1 (-2.4 to 6.6)	0.47	-1.3 (-8.7 to 6.0)	0.39

**Supplemental Table S3: Clinical and socio-demographic characteristics of the participants in the total group, in the ENVIRONAGE birth cohort, and reference values from Flanders.** Population characteristics of the current study (n = 463), the entire ENVIRONAGE Cohort (n = 814), and the reference population as births in Flanders (born in 2002 until 2011; n = 606,877). Data is presented as median (10th percentile – 90th percentile) for continuous variables, and as n (%) for discrete variables.

Characteristics	Current study n = 463	ENVIRONAGE n = 814	Births in Flanders (1) n = 606,877
<b>Maternal variables</b>			
Age, years	29.0 (23.0 – 35.0)	29.0 (23.0 – 35.0)	29.5 (23.5 – 35.8)
Pre-pregnancy BMI <sup>a</sup> , kg/m <sup>3</sup>	23.2 (19.5 – 30.1)	23.4 (19.6 – 30.7)	N/A
<i>Self-reported tobacco use</i>			
Never smokers	288 (62%)	512 (63%)	N/A
Smoked before pregnancy	115 (25%)	188 (23%)	N/A
Smoked during pregnancy	60 (13%)	113 (14%)	N/A
<i>Alcohol consumption during pregnancy</i>			
No	387 (83%)	664 (83%)	N/A
≤ 1 glass a day	68 (15%)	117 (15%)	N/A
> 1 glass a day	8 (2%)	13 (12%)	N/A
<i>Maternal education<sup>b</sup></i>			
Low	47 (10%)	95 (12%)	58,743 (13%)
Middle	170 (37%)	290 (36%)	183,410 (41%)
High	246 (53%)	418 (52%)	5,968 (46%)
<b>Newborn variables</b>			
Gestational age, weeks	39.0 (38.0 – 41.0)	39.0 (37.0 – 41.0)	N/A
Birth weight, g	3,390 (2,845 – 3,965)	3,385 (2,805 – 4,005)	3,360 (2,740 – 3,965)
Birth length <sup>a</sup> , cm	50.5 (48.0 – 53.0)	50.0 (48.0 – 53.0)	N/A
<i>Gender</i>			
Male	234 (51%)	402 (49%)	311,620 (51%)
Female	229 (49%)	412 (51%)	295,257 (49%)
<i>Ethnicity<sup>c</sup></i>			
European	415 (90%)	715 (88%)	384,522 (88%)
Non-European	48 (10%)	99 (12%)	222,355 (12%)
<i>Season at birth</i>			
Winter (Dec – Mar)	125 (27%)	238 (29%)	147,471 (24%)
Spring (Mar – Jun)	108 (23%)	165 (21%)	152,326 (26%)
Summer (Jun – Sep)	108 (23%)	180 (22%)	157,788 (26%)
Autumn (Sep – Dec)	122 (27%)	231 (28%)	149,292 (24%)

<sup>a</sup> n = 807 for the ENVIRONAGE Cohort

<sup>b</sup> Maternal education was coded as ‘low’ (no diploma or primary school), ‘middle’ (high school) and ‘high’ (college or university degree).

<sup>c</sup> Ethnicity is classified based on the native country of the newborns’ grandparents as either ‘European’ (at least two grandparents were European) or ‘non-European’ (at least three grandparents were of non-European origin).

N/A = Not Available

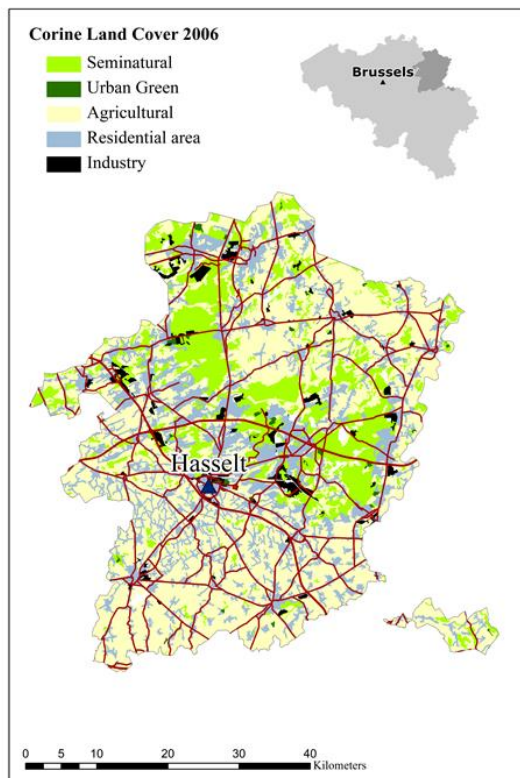
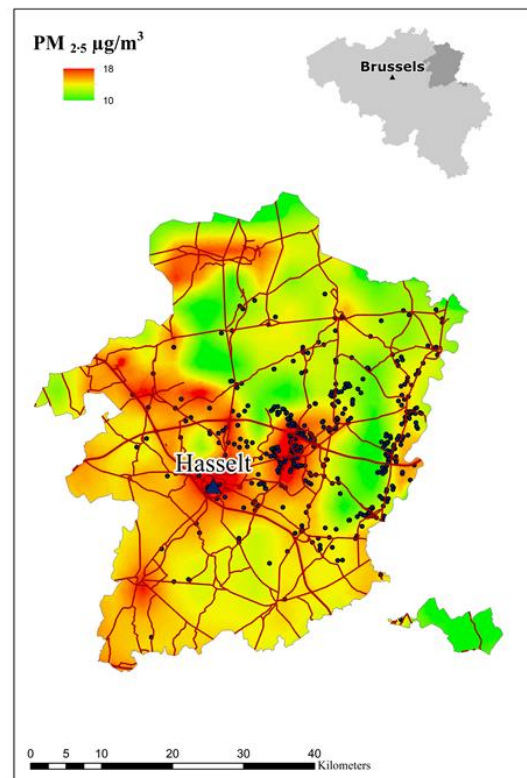
**Supplemental Table S4: DNA methylation information.** Mean  $\pm$  SD of the specific CpG sites for *APEX1* (n = 3), *OGG1* (n = 3), *PARP1* (n = 7), *ERCC1* (n = 4), *ERCC4* (n = 5), *p53* (n = 5), and *DAPK1* (n = 3) are presented. Average methylation of the investigated promoter region per gene is indicated in bold.

DNA methylation levels (%)	Mean $\pm$ SD
<b><i>APEX1</i></b>	<b>1.3 <math>\pm</math> 0.9</b>
<i>CpG 1</i>	0.8 $\pm$ 0.7
<i>CpG 2</i>	1.3 $\pm$ 0.8
<i>CpG 3</i>	1.9 $\pm$ 0.7
<b><i>OGG1</i></b>	<b>0.8 <math>\pm</math> 0.9</b>
<i>CpG 1</i>	0.4 $\pm$ 0.5
<i>CpG 2</i>	1.6 $\pm$ 0.9
<i>CpG 3</i>	0.6 $\pm$ 0.8
<b><i>PARP1</i></b>	<b>1.9 <math>\pm</math> 2.4</b>
<i>CpG 1</i>	2.8 $\pm$ 3.0
<i>CpG 2</i>	0.5 $\pm$ 0.9
<i>CpG 3</i>	0.8 $\pm$ 1.3
<i>CpG 4</i>	2.1 $\pm$ 1.8
<i>CpG 5</i>	1.9 $\pm$ 2.4
<i>CpG 6</i>	0.4 $\pm$ 1.1
<i>CpG 7</i>	4.5 $\pm$ 2.1
<b><i>ERCC1</i></b>	<b>1.7 <math>\pm</math> 2.3</b>
<i>CpG 1</i>	1.1 $\pm$ 2.0
<i>CpG 2</i>	4.1 $\pm$ 2.3
<i>CpG 3</i>	0.5 $\pm$ 1.4
<i>CpG 4</i>	1.0 $\pm$ 1.4
<b><i>ERCC4</i></b>	<b>1.8 <math>\pm</math> 2.3</b>
<i>CpG 1</i>	2.1 $\pm$ 2.3
<i>CpG 2</i>	2.8 $\pm$ 2.7
<i>CpG 3</i>	1.1 $\pm$ 1.9
<i>CpG 4</i>	1.3 $\pm$ 1.8
<i>CpG 5</i>	1.5 $\pm$ 2.3
<b><i>p53</i></b>	<b>4.9 <math>\pm</math> 3.6</b>
<i>CpG 1</i>	4.6 $\pm$ 2.9
<i>CpG 2</i>	9.1 $\pm$ 4.3
<i>CpG 3</i>	3.3 $\pm$ 1.6
<i>CpG 4</i>	2.6 $\pm$ 2.1
<i>CpG 5</i>	4.9 $\pm$ 1.8
<b><i>DAPK1</i></b>	<b>1.1 <math>\pm</math> 1.1</b>
<i>CpG 1</i>	0.8 $\pm$ 0.8
<i>CpG 2</i>	0.7 $\pm$ 0.8
<i>CpG 3</i>	1.8 $\pm$ 1.2

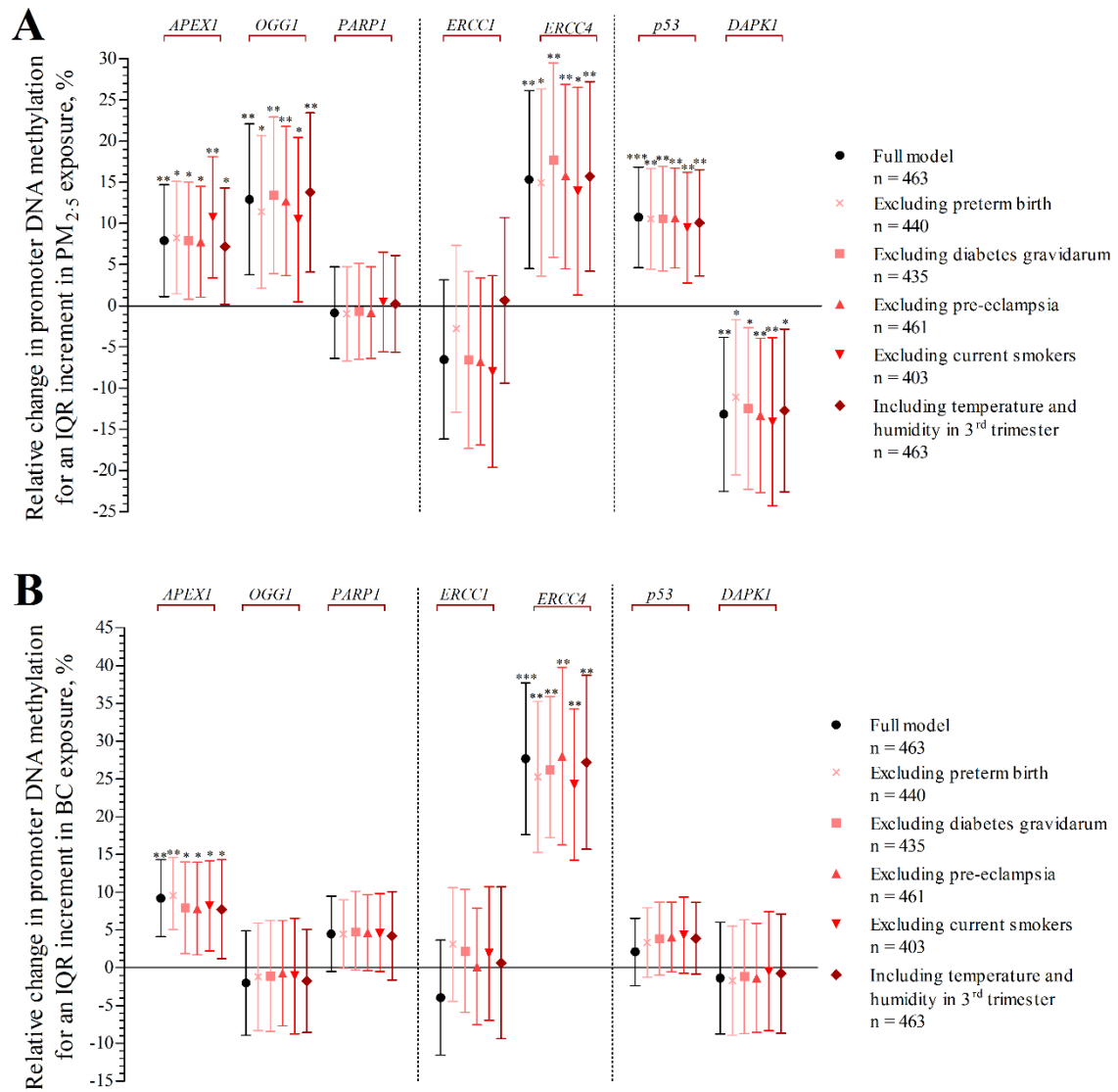
**Supplemental Table S5: Association between mutation marker, and DNA repair genes in association with PM<sub>10</sub>, PM<sub>2.5</sub>, black carbon (BC), and NO<sub>2</sub> exposure during the entire pregnancy.** All models were adjusted for the following covariates: newborns' sex, ethnicity, parity, maternal age, maternal education, smoking habits, pre-pregnancy BMI, gestational age, season at delivery, and batch effect. In order to obtain a relevant exposure change, the effect estimates of the mutation marker and genes were presented as a relative percentage change in methylation level for an interquartile range (IQR) increment in air pollution exposure during the entire pregnancy: 4.41 µg/m<sup>3</sup> for PM<sub>10</sub>, 3.84 µg/m<sup>3</sup> for PM<sub>2.5</sub>, 0.36 µg/m<sup>3</sup> for BC, and 5.34 µg/m<sup>3</sup> for NO<sub>2</sub>.

	PM <sub>10</sub>		PM <sub>2.5</sub>		BC		NO <sub>2</sub>	
	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value
<i>APEXI</i>	6.3 (0.2 to 12.3)	0.042	9.0 (15.7 to 2.3)	0.0089	9.2 (4.1 to 14.2)	0.0005	1.3 (-3.2 to 5.9)	0.57
<i>OGGI</i>	8.0 (0.1 to 16)	0.048	13.9 (22.6 to 5.1)	0.0054	-2.0 (-8.9 to 4.9)	0.56	1.9 (-4.1 to 7.9)	0.53
<i>PARP1</i>	0.6 (-4.6 to 5.7)	0.83	-0.5 (5 to -6.1)	0.85	4.5 (-0.5 to 9.4)	0.29	3.2 (-0.5 to 6.9)	0.091
<i>ERCC1</i>	-8.6 (-17.9 to 0.6)	0.15	-6.2 (3.6 to -16)	0.22	-3.9 (-11.5 to 3.6)	0.31	-2.5 (-8.8 to 3.9)	0.44
<i>ERCC4</i>	22.5 (10.7 to 34.4)	<0.0001	16.3 (27.2 to 5.4)	0.0034	27.6 (17.6 to 37.6)	<0.0001	8.0 (-1.0 to 17.1)	0.082
<i>p53</i>	5.4 (0 to 10.6)	0.046	10.6 (16.7 to 4.5)	0.0008	2.1 (-2.4 to 6.5)	0.36	1.4 (-2.6 to 5.5)	0.48
<i>DAPK1</i>	-7.4 (-15.5 to 0.8)	0.076	-12.9 (-3.5 to -22.4)	0.0073	-1.3 (-8.7 to 6)	0.72	-3.9 (-10.1 to 2.2)	0.21
<i>Alu</i>	23.0 (0.6 to 3.9)	0.042	3.0 (5.7 to 0.2)	0.035	2.0 (1.4 to 2.5)	0.0007	0.7 (-0.9 to 2.4)	0.40



**A****B**

**Supplemental Figure S1. Maps of the province of Limburg (Flanders, Belgium).** In Panel A, the land use, according to the Corine Land Cover data-set: Semi-natural, Agricultural, Urban Green, Residential Area, and Industry. In Panel B, the yearly interpolated  $PM_{2.5}$  concentrations, and the mothers' residence (black dots) in the recruitment area. Bold red lines represent highways, thin red lines represent major roads.



**Supplemental Figure S2. Sensitivity analysis.** The associations between relative methylation in placental DNA repair genes and air pollution exposure were re-evaluated after excluding preterm births (Excluding preterm birth; n = 440) mothers with gestation diabetes (Excluding diabetes gravidarum; n = 435), excluding mothers with pre-eclampsia, i.e. pregnancy disorder characterised by the onset of high blood pressure and proteinuria (Excluding pre-eclampsia; n = 461), and mothers who smoked during pregnancy (Excluding current smokers; n = 403). All models were adjusted for newborn's sex, ethnicity, maternal age, education, smoking habits, pre-pregnancy BMI, gestational age, season at delivery, and batch effect. The apparent mean temperature (°C) and relative humidity during the third trimester were also added in the sensitivity model (Including temperature and humidity in 3<sup>rd</sup> trimester; n = 463). All estimates are presented as a relative percentage change in methylation level for an IQR increment of 3.84 µg/m<sup>3</sup> PM<sub>2.5</sub> exposure during the entire pregnancy (Panel A), and for an IQR increment 0.36 µg/m<sup>3</sup> black carbon (BC) exposure during the entire pregnancy (Panel B).  
 \* p < 0.05; \*\* p < 0.01; \*\*\* p < 0.0001

## REFERENCES

1. Cox B, Martens E, Nemery B, Vangronsveld J, Nawrot TS. Impact of a stepwise introduction of smoke-free legislation on the rate of preterm births: analysis of routinely collected birth data. *BMJ*. 2013;346:f441.