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Supplemental Material

***In Utero* Fine Particle Air Pollution and Placental Expression of Genes in the Brain-Derived Neurotrophic Factor Signaling Pathway: An ENVIRONAGE Birth Cohort Study**

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age, maternal education, gestational age, cord blood insulin, placental biopsy site, delivery date, season at birth and NO₂ exposure. * p < 0.05

Table S1. Primer assays for selected genes and their RefSeq number.

Abbreviation	Gene	RefSeq number	Primetime [®] Std qPCR Assay	Efficiency (%)
Target genes				
<i>BDNF</i>	Brain-derived neurotrophic factor	NM_001709	Hs.PT.56a.27098180.g	100
<i>TRKB</i>	Neurotrophic tyrosine kinase receptor type 2	NM_001018065	Hs.PT.56a.39058236.g	112
<i>AKT1</i>	V-akt murine thymoma viral oncogene homolog 1	NM_005163	Hs.PT.56a.15697853.g	92
<i>AKT2</i>	V-akt murine thymoma viral oncogene homolog 2	NM_001243027	Hs.PT.56a.143554.g	99
<i>AKT3</i>	V-akt murine thymoma viral oncogene homolog 3	NM_005465	Hs.PT.56a.4178001	92
<i>SOS1</i>	Son of sevenless homolog 1	NM_005633	Hs.PT.56a.20852108	93
<i>SOS2</i>	Son of sevenless homolog 2	NM_006939	Hs.PT.56a.14433335	92
<i>PLCG1</i>	Phospholipase C gamma 1	NM_182811	Hs.PT.56a.2187691	99
<i>PLCG2</i>	Phospholipase C gamma 2	NM_002661	Hs.PT.56a.45287498	99
<i>SYN1</i>	Synapsin 1	NM_006950	Hs.PT.56a.4883078.g	107
Reference genes				
<i>GAPDH</i>	Glyceraldehyde-3-phosphate dehydrogenase	NM_001256799	Hs.PT.53a.24391631.gs	100
<i>IPO8</i>	Importin 8	NM_001190995	Hs.PT.56a.40532361	95
<i>UBC</i>	Ubiquitin C	NM_021009	Hs.PT.39a.22214853	95
<i>POLR2A</i>	Polymerase (RNA) II, polypeptide A	NM_000937	Hs.PT.56a.25515089	95

Table S2. Within-placenta and between-placenta variability of the two placental biopsies for each gene.

Gene	Within-placenta variability (%)	Between-placenta variability (%)
<i>BDNF</i>	38.7	61.3
<i>TRKB</i>	42.3	57.7
<i>AKT1</i>	16.1	83.9
<i>AKT2</i>	52.7	47.3
<i>AKT3</i>	39.0	61.0
<i>SOS1</i>	43.7	56.3
<i>SOS2</i>	38.9	61.1
<i>PLCG1</i>	30.2	69.8
<i>PLCG2</i>	53.3	46.7
<i>SYN1</i>	69.3	30.7

Table S3. Correlations between genes in the *Bdnf* signaling pathway.

	<i>BDNF</i>	<i>TRKB</i>	<i>AKT1</i>	<i>AKT2</i>	<i>AKT3</i>	<i>SOS1</i>	<i>SOS2</i>	<i>PLCG1</i>	<i>PLCG2</i>	<i>SYN1</i>
<i>BDNF</i>	1	0.071 0.43	-0.48 <.0001	0.071 0.39	-0.23 0.004	0.18 0.03	0.43 <.0001	-0.43 <.0001	0.19 0.02	0.19 0.03
<i>TRKB</i>		1	-0.07 0.42	-0.04 0.63	-0.027 0.75	-0.0069 0.94	0.058 0.50	-0.034 0.70	-0.021 0.82	0.16 0.08
<i>AKT1</i>			1	0.33 <.0001	0.42 <.0001	-0.10 0.21	-0.40 <.0001	0.74 <.0001	-0.13 0.12	-0.02 0.77
<i>AKT2</i>				1	0.65 <.0001	0.48 <.0001	0.27 0.0004	0.28 0.0002	0.43 <.0001	0.28 0.0005
<i>AKT3</i>					1	0.39 <.0001	0.18 0.018	0.36 <.0001	0.049 0.54	0.16 0.05
<i>SOS1</i>						1	0.60 <.0001	0.13 0.096	0.54 <.0001	0.08 0.33
<i>SOS2</i>							1	-0.30 <.0001	0.26 0.0012	-0.04 0.67
<i>PLCG1</i>								1	0.15 0.068	0.07 0.39
<i>PLCG2</i>									1	0.31 0.0002
<i>SYN1</i>										1

Given is pearson correlation and p-value.

Table S4. Exposure characteristics of NO₂ (n = 90).

Time windows	NO ₂ ,ug/m ³		
	Mean ± SD	25 th percentile	75 th percentile
Pre-implantation (1-5d)	21.6 ± 8.9	15.8	25.5
Implantation (6-12d)	21.1 ± 8.1	15.6	25.6
Implantation range ^a (6-21d)	21.0 ± 7.2	15.8	25.0
Post-implantation (22-28d)	19.9 ± 8.2	13.3	24.1
First month (1-30d)	20.4 ± 7.1	15.3	24.4
Trimester 1 (1-13w)	20.3 ± 6.6	15.9	24.3
Trimester 2 (14-26w)	22.3 ± 7.2	16.6	26.4
Trimester 3 (27w-delivery)	23.6 ± 7.3	18.3	28.1

^aData available for 79 subjects.

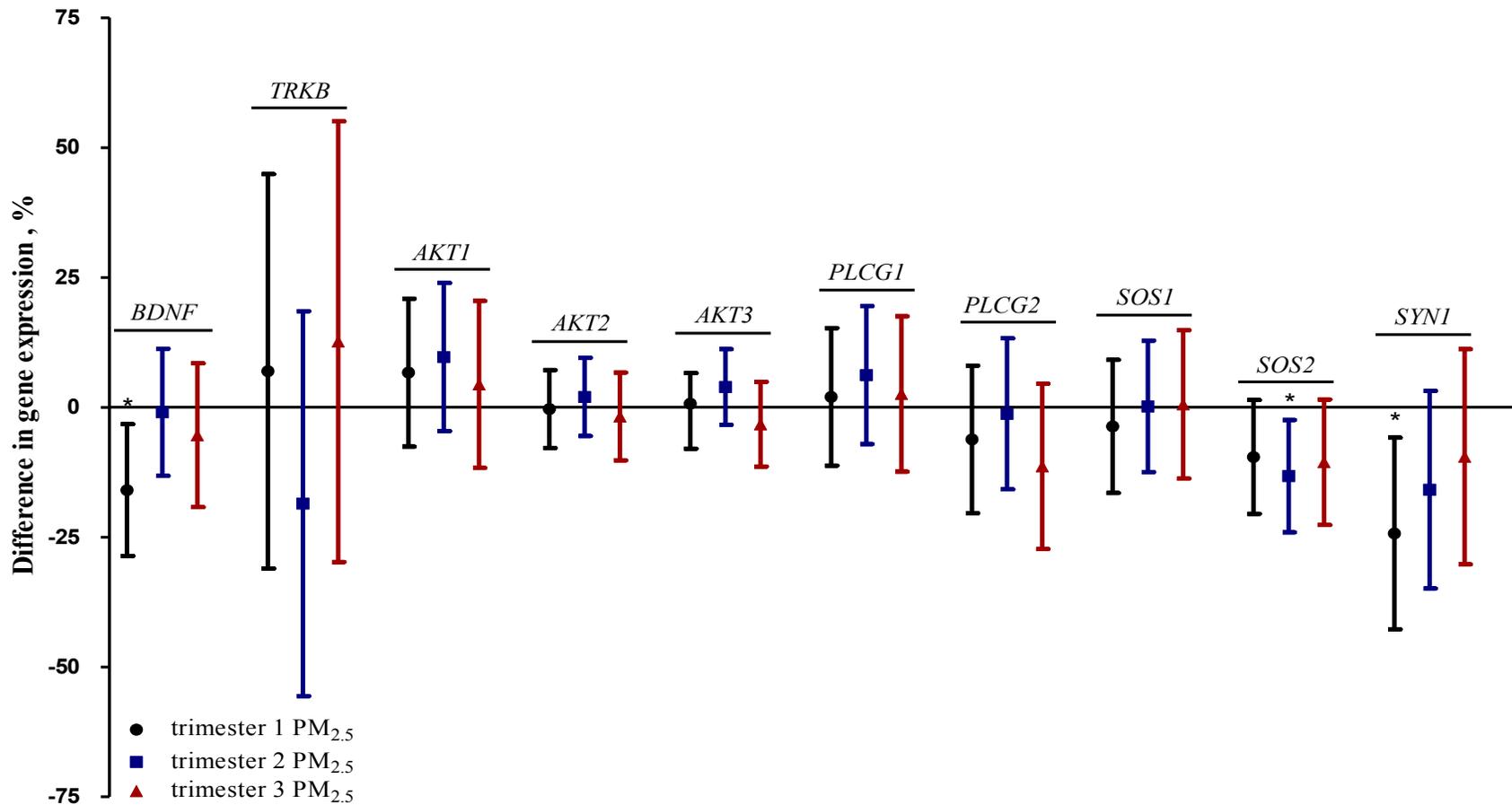


Figure S1. Difference in placental gene expression in association with *in utero* exposure to fine particle air pollution (PM_{2.5}) during various time windows (single-gene models; n=90). The effect estimates are the percent difference (95% CI) relative to mean gene expression for a 5 $\mu\text{g}/\text{m}^3$ increment of PM_{2.5} exposure ($\mu\text{g}/\text{m}^3$). Time window specific PM_{2.5} exposures ($\mu\text{g}/\text{m}^3$) were calculated by averaging the daily interpolated PM_{2.5} concentrations for various periods during pregnancy: each of the three trimesters. Estimates were adjusted for newborn's gender, maternal age, maternal education, gestational age, cord blood insulin, placental biopsy site, delivery date, season at birth and NO₂ exposure. * p < 0.05