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Supplementary Materials for

Impact of air pollution exposure on cytokines and histone modification profiles at single-cell levels during pregnancy

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Fig. S1. Location of the study population and the EPA central monitoring stations with 5km and 10km perimeters and PM_{2.5} validation

A. The map illustrates the residential locations of the study population and Environmental Protection Agency (EPA) Central ambient air pollution monitoring stations with 5km and 10km perimeters. Study participants are marked with red dots, while EPA stations are marked with black dots. Light blue and dark blue areas indicate the radius around EPA stations at 5km and 10km, respectively. The map shows that most study participants are located within 10km of the EPA monitoring stations.

B. The Pearson correlation plot between our 3-month average $PM_{2.5}$ estimates from the Inverse Distance Weighting method (IDW) and 3-month average $PM_{2.5}$ from the resolution of 1km grids data set (60, 57) indicates our $PM_{2.5}$ estimates are consistent with the high-resolution spatial PM estimates. The x-axis represents the $PM_{2.5}$ estimates ($\mu g/m^3$) from the IDW used in the manuscript, while the y-axis shows the $PM_{2.5}$ estimates from Di et al. (57). The red dashed line indicates the 45-degree line.



A. 1- week prior to blood collection exposure window

B. 6-months prior to blood collection exposure window

2.0

PM2.5 (1µg/m3)



C. 9-months prior to blood collection exposure window



Fig. S2. The association between the different PM2.5 exposure windows and cytokine levels

Volcano plots summarize the β coefficients and statistical significance for the association between marker levels and the average $PM_{2.5}$ levels (ug/m³) by pregnant status. For the sensitivity analysis, we used the different exposure windows, including (1) 1-week, (2) 6-month, (3) 9-month prior to blood collection. Multivariable regression was used to estimate the differential impact of PM_{2.5} levels on the immune markers between pregnant verse. Nonpregnant women. The level of cytokine in the blood sample is a function of the PM2.5 exposure level (µg/m3), pregnant status, the interaction between PM2.5 and pregnant status, age, ethnicity (Hispanic vs. not Hispanic), asthma status, and zipcode-level social deprivation index (SDI) score. Multiple testing corrections were performed by using FDR ≥ 0.05 . A β coefficient for the interaction between PM_{2.5} and pregnant status represents the relationship between cytokine levels and PM_{2.5} levels, comparing pregnant and non-pregnant women. A β coefficient of PM_{2.5} indicates that cytokine levels will change by β when PM_{2.5} increases by 1 µg/m³. A positive β coefficient for pregnant status represents the β difference in cytokine levels between pregnant and non-pregnant individuals.



Fig. S3. Impact of PM_{2.5} exposure on neonatal-maternal cytokine associations by different exposure windows and cutoff points for sensitivity analysis

(A)-(C): Volcano plots summarize the β coefficients and statistical significance for the association between maternal marker levels and cord blood (CB) markers, presented by the average PM_{2.5} exposure window during the (A) 1 week prior to CB collection, as well as during the (B) first, and (C) second trimester.

(D): To check the robustness of our main analysis (Figure 3), different cutoff points were used to dichotomize the $PM_{2.5}$ exposure group, categorizing values below the 25th percentile as low exposure and values above the 25th percentile as high exposure.

(A)-(D): Multivariable regression was used to estimate the impact of prenatal PM_{2.5} levels on the immune biomarkers in 33 CB samples. The level of cytokine in CB is a function of the level of matching cytokines in CB's mother, PM_{2.5} exposure group (low vs. high; the cutoff point is 12 ug/m³), the interaction between the mother's matching cytokine level and PM_{2.5}, mother's age, ethnicity (Hispanic vs. not Hispanic), asthma status, and SDI score. Multiple testing corrections were performed by using FDR at 0.05. A positive β coefficient can be interpreted 1 unit increase in maternal cytokine will increase the cytokines level in CB by β estimates. A β coefficient of PM_{2.5} (binary indicator; low vs. high) can be interpreted that cytokine levels in CB will change by β among high prenatal PM_{2.5} exposure compared to the low exposure group. A β coefficient

for the interaction between maternal cytokine levels and $PM_{2.5}$ status represents the relationship between maternal cytokine levels and the corresponding cytokine levels in cord blood, comparing high maternal $PM_{2.5}$ exposure to low maternal $PM_{2.5}$ exposure.



Fig. S4. Single-cell UMAP colored by cell type identity for each acetylation and methylation panel.

Uniform Manifold Approximation and Projection (UMAPs) were shown using cell surface markers for both the acetylation (A) and methylation (B) panels to test whether cell identities were similar between both panels. In general, similar cell identities were confirmed.

Acetylation Panel







D. Exposure window: 9-months



Fig. S5 Impact of PM_{2.5} on the acetylation panel Histone post-translational modifications (HPTMs) in 11 immune cells depending on pregnancy status

Heatmaps summarize the beta (β) coefficients and FDR-adjusted p-values (Q-values) (<0.05) from the multivariable mixed-effect model adjusted for covariates, including age and asthma status (see method section for further details). Each row of heatmaps represents the different exposure windows, including 1-week, 3-months, 6-months, and 9-months prior to the blood collection. The x-axis of the heatmap indicates the 18 markers from the acetylation panel, and the y-axis shows the 11 immune cell types. The first column presents the differentiated effect of PM_{2.5} on pregnancy status (the interaction term between PM_{2.5} and pregnant status), the second column shows the changes in HPTM levels between pregnant and non-pregnant women, and the last column presents the size of the coefficient, in which red represents the positive coefficients and blue shows the negative coefficients. Significant coefficients are highlighted with significant levels. Q-values below 0.05 get one star (*); below 0.01, two stars (**); and below 0.001, three stars (***). Descriptions for the abbreviations of HPTMs can be found in Table S2.

Methylation Panel

A. Exposure window: 1-week









D. Exposure window: 9-months



Fig. S6 Impact of PM_{2.5} on the methylation panel Histone post-translational modifications (HPTMs) in 11 immune cells depending on pregnancy status

Heatmaps summarize the beta (β) coefficients and FDR-adjusted p-values (Q-values) (<0.05) from the multivariable mixed-effect model adjusted for covariates, including age and asthma status, (see method section for further details). Each row of heatmaps represents the different exposure windows, including 1-week, 3-months, 6-months, and 9-months prior to the blood collection. The x-axis of the heatmap indicates the 20 markers from the methylation panel, and the y-axis shows the 11 immune cell types. The first column presents the differentiated effect of PM_{2.5} on pregnancy status (the interaction term between PM_{2.5} and pregnant status), the second column shows the changes in HPTM levels between pregnant and non-pregnant women, and the last column presents the size of the coefficient, in which red represents the positive coefficients and blue shows the negative coefficients. Significant coefficients are highlighted with significant levels. Q-values below 0.05 get one star (*); below 0.01, two stars (**); and below 0.001, three stars (***). Descriptions for the abbreviations of HPTMs can be found in Table S2.



Fig. S7. Principal component analysis (PCA) of cytokine and Histone Post-Translational Modifications (HPTM) data

A. PCA for cytokine data: The *eigencor* plot demonstrates whether there is an effect of race/ethnicity, pregnant status (pregnant vs. non-pregnant), asthma status (asthma vs. no asthma), batch effect, PM_{2.5} exposure level (ug/m3), age or zipcode-level social-deprivation index (SDI) on the principal components of the data. Significant effects were observed in race/ethnicity and pregnant status, and PM2.5 exposure level (PC1), asthma status and age (PC2), batch effect (PC3), age (PC4), and batch effect (PC5). After adjusting for the batch effect using combat, the second *eigencor* plot shows effects race/ethnicity, pregnant status, and PM2.5 exposure level (PC1). We include ethnicity, asthma status, and SDI score in the statistical model as potential confounding. The scale bar represents Eigenvalues (-1 to 1, with 0 having no correlation). P-values below 0.05 get one star (*); below 0.01, two stars (**); and below 0.001, three stars (***).

B. PCA for HPTM data: The *eigencor* plots demonstrate whether there is an effect of race/ethnicity, pregnant status (pregnant vs. non-pregnant), asthma status (asthma vs. no asthma), batch effect, $PM_{2.5}$ exposure level (low vs. high), age or zipcode-level social-deprivation index on the principal components for acetylation and methylation HPTM data, respectively. We include the scale bar representing Eigenvalues (- 1 to 1, with 0 having no correlation). P-values below 0.05 are shown by one asterisk (*); below 0.01, two (**); and below 0.001, three (***).

Type of participants	Exposure windows	Luminex	EpiTOF
	11	(average, se)	(average, se)
Pregnant women	collection (ug/m ³)	12.5 (0.44)	12.5 (1.00)
	Low exposure group (%)	63.0	63.9
	3-months prior blood collection (ug/m^3)	13.7 (0.35)	14.0 (1.04)
	Low exposure group (%)	42.4	50
	6-months prior blood collection (ug/m^3)	13.7 (0.26)	14.5 (0.621)
	Low exposure group (%)	41.8	36.1
	9-months prior blood collection (ug/m ³)	14.1 (0.13)	14.4 (0.33)
	Low exposure group (%)	12.1	5.56
Non-pregnant women	1-week prior blood collection (ug/m ³)	11.7 (0.97)	13.0 (1.64)
	Low exposure group (%)	75.2	52.4
	3-months prior blood collection (ug/m^3)	9.80 (0.22)	11.8 (0.79)
	Low exposure group (%)	73.8	42.9
	6-months prior blood collection (ug/m^3)	9.20 (0.36.415)	10.3 (0.49)
	Low exposure group (%)	83.4	76.2
	9-months prior blood collection (ug/m^3)	8.82 (0.15)	9.27 (0.34)
	Low exposure group (%)	93.1	100
Neonates	1-week prior CB collection (ug/m ³)	12.64 (1.09)	
	Low exposure group (%)	21.2	
	1^{st} trimester collection (ug/m^3)	13.81 (0.57)	
	Low exposure group (%)	36.3	
	2^{nd} trimester collection (ug/m ³)	14.05 (0.79)	
	Low exposure group (%)	36.3	
	3^{rd} trimester collection (ug/m ³)	13.81 (0.57)	
	Low exposure group (%)	36.3	

Table S1. The average PM2.5 concentration levels by exposure windows

Note: The low exposure group was defined as those with average $PM_{2.5}$ exposures below the 2012 EPA National Ambient Air Quality Primary Standard of 12 µg/m³ (details are provided in the Methods section). The EPA standard does not imply that levels lower than the standards are safe. The pregnancy trimesters were calculated using the date of the CB collection and the number of weeks of gestation on the date of the maternal blood collection.

Table S2. Histone Modification and their abbreviations for Panel 1 (Acetylation) and Panel2 (Methylation)

Acetylation panel		Methylation panel		
Abbreviation	Description	Abbreviation	Description	
H2BK120ub	Histone H2B ubiquitination at lysine 120	macroH2A	a variant of the histone H2A	
CrotonylK	Lysine crotonylation	H3K4me3	Histone H3 trimethylation at lysine 4	
H3R2cit	Histone H3 citrullination at arginine 2	H2A.Z	variant of the histone H2A	
H3K14ac	Histone H3 acetylation at lysine 14	H3K36me1	Histone H3 monomethylation at lysine 36	
H4K16ac	Histone H4 lysine 16 acetylation	H4K20me1	Histone H4 monomethylation at lysine 20	
H3K23ac	Histone H3 acetylation at lysine 23.	H3K27me1	Histone H3 monomethylation at lysine 27	
H2BS14ph	Histone H2B phosphorylation at serine 14	H3K36me2	Histone H3 dimethylation at lysine 36	
H3K18ac	Histone H3 acetylation at lysine 18	H4K20me2	Histone H4 dimethylation at lysine 20	
H3K56ac	Histone H3 acetylation at lysine 56	H3.3	A variant of the histone H3	
AcetylK	Acetylation at lysine residues	H4K20me3	Histone H4 trimethylation at lysine 20	
gamma(γ)H2AX	Phosphorylation of histone H2A variant H2AX at serine 139	H3K27me3	Histone H3 trimethylation at lysine 27	
H2BK5ac	Histone H2B acetylation at lysine 5	Rme1	Arginine monomethylation	
H3S10ph	Histone H3 phosphorylation at serine 10	Rme2sym	symmetric arginine dimethylation	
H4K5ac	Histone H4 acetylation at lysine 5	H3K4me2	Histone H3 dimethylation at lysine 4	
CleavedH3T22	Proteolytic cleavage of H3 histone at threonine 22	H3K36me3	Histone H3 trimethylation at lysine 36	
H3K9ac	Histone H3 acetylation at lysine 9	H3K9me2	Histone H3 dimethylation at lysine 9	
H2AK119Ub	Histone H2A lysine 119 ubiquitination	H3K9me1	Histone H3 monomethylation at lysine 9	
H3K27ac	Histone H3 acetylation at lysine 27	Rme2asy	Asymmetric arginine dimethylation	
		H3K27me2	Histone H3 dimethylation at lysine 27	
		CENPA	A variant of histone H3 found at the centromere	

Panel1					Panel2				
Metal	Marker	Manufacturer	Туре	Clone	Metal	Marker	Manufacturer	Туре	Clone
89Y	CD45	Fluidigm	Mouse IgG1	HI30	89Y	CD45	Fluidigm	Mouse IgG1	HI30
141Pr	H3	CST	Rabbit mAb	D1H2	141Pr	H3	CST	Rabbit mAb	D1H2
142Nd	γ-H2AX	CST	Rabbit mAb	20E3	142Nd	Arg-me1	Abcam	Mouse IgG1	5D1
143Nd	H2BK5ac	CST	Rabbit mAb	D5H1S	143Nd	Arg-me2 (sym)	CST	Rabbit mAb	Unknown
144Nd	H3S10ph	Active Motif	Mouse IgG1	MABI 0312	144Nd	H3K4me2	Active Motif	Mouse IgG1	MABI 0303
145Nd	CD4	BioLegend	Mouse IgG1	RPA-T4	145Nd	CD4	BioLegend	Mouse IgG1	RPA-T4
146Nd	CD8	BioLegend	Mouse IgG1	SK1	146Nd	CD8	BioLegend	Mouse IgG1	SK1
147Sm	H4K5ac	Active Motif	Mouse IgG1	MABI 0405	147Sm	H3K9me2	BioLegend	Mouse IgG1	5E5-G5
148Nd	CD34	BioLegend	Mouse IgG1	581	148Nd	CD34	BioLegend	Mouse IgG1	581
149Sm	Cleaved H3 (Thr22)	CST	Rabbit mAb	D7J2K	149Sm	H3K9me1	Biolegend	Mouse IgG1	7E7.H12
151Eu	H3K23ac	RevMab	Rabbit mAb	RM169	150Nd	H3K36me3	RevMab	Rabbit mAb	RM155
152Sm	H3K9ac	Active Motif	Mouse IgG2a	2G1F9	151Eu	H3K27me1	Active Motif	Mouse IgG2a	MABI 0321
153Eu	H2BS14ph	CST	Rabbit mAb	D67H2	152Sm	Arg-me2 (asy)	CST	Rabbit mAb	Unknown
154Sm	H2AK119ub	CST	Rabbit mAb	D27C4	153Eu	H3K36me2	Active Motif	Mouse IgG1	MABI 0332
155Gd	CD11c	BioLegend	Mouse IgG1	Bu15	154Sm	H3K27me2	Active Motif	Mouse IgG2a	MABI 0324
156Gd	H3K18ac	RevMAb	Rabbit mAb	RM166	155Gd	CD11c	BioLegend	Mouse IgG1	Bu15
158Gd	H3K56ac	Active Motif	Mouse IgG1	12.1	156Gd	H4K20me2	Active Motif	Mouse IgG2a	MABI 0422
159Tb	CD14	BioLegend	Mouse IgG2a	M5E2	158Gd	H3.3	Abcam	Rabbit mAb	EPR17899
160Gd	PADI4	OriGene	lgG2a	OTI4H5	159Tb	CD14	BioLegend	Mouse IgG2a	M5E2
161Dy	H2BK120ub	CST	Rabbit mAb	D11	160Gd	H4K20me3	BioLegend	Mouse IgG1	6F8-D9
163Dy	H3R2cit	Abcam	Rabbit mAb	EPR17703	161Dy	Macro-H2A	Millipore	Mouse IgG2b	14G7
164Dy	H3K14ac	CST	Rabbit mAb	D4B9	162Dy	H3K4me3	Life	Rabbit IgG	G.532.8
166Er	CD33	BioLegend	Mouse IgG1	WM53	163Dy	H2A.Z			
167Er	CD16	BioLegend	Mouse IgG1	B73.1	164Dy	H3K36me1	Abcam	Rabbit mAb	EPR16993
168Er	H4K16ac	CST	Rabbit mAb	E2B8W	165Ho	H3K27me3	Active Motif	Mouse IgG1	MABI 0323
169Tm	CD123	BD	Mouse IgG1	9F5	166Er	CD33	BioLegend	Mouse IgG1	WM53
170Er	CD3	BioLegend	Mouse IgG1	UCHT1	167Er	CD16	BioLegend	Mouse IgG1	B73.1
171Yb	CD38	Biolegend	Mouse IgG1	HIT2	168Er	H4K20me1	Active Motif	Mouse IgG	5E10-D8
172Yb	CD56	BD	Mouse	NCAM16.2	169Tm	CD123	BD	Mouse IgG1	9F5
173Yb	H4	Abcam	Mouse IgG1	ab31830	170Er	CD3	BioLegend	Mouse IgG1	UCHT1
174Yb	H3K27ac	Active Motif	Mouse IgG1	MABI 0309	171Yb	CD38	Biolegend	Mouse IgG1	HIT2
175Lu	CD19	BioLegend	Mouse IgG1	HIB19	172Yb	CD56	BD	Mouse IgG2b	NCAM16.2
176Yb	HLA-DR	BioLegend	Mouse IgG2a	L243	173Yb	H4	Abcam	Mouse IgG1	ab31830
					174Yb	CENP-A	MBL	Mouse IgG1	19-Mar
					175Lu	CD19	BioLegend	Mouse IgG1	HIB19
					176Yb	HLA-DR	BioLegend	Mouse IgG2a	L243

Table S3. EpiTOF Antibody for Panel 1 (Acetylation) and Panel 2 (Methylation)

Table S4. Antibody Sources

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies	•	
Rabbit monoclonal anti-Histone H3 (clone D1H2)	Cell Signaling Technology	4499 (custom formulation*)
Rabbit monoclonal anti-phospho-Histone H2A.X (Ser139) (clone 20E3)	Cell Signaling Technology	9718 (custom formulation*)
Rabbit monoclonal anti-acetyl-Histone H2B (Lys5) (clone D5H1S)	Cell Signaling Technology	12799 (custom formulation*)
Mouse monoclonal anti-phospho-Histone H3 (Ser10) (clone MABI 0312)	Active Motif	39636
Mouse monoclonal anti-acetyl-Histone H4 (Lys5) (clone MABI 0405)	Active Motif	61523
Rabbit monoclonal anti-cleaved-Histone H3 (Thr22) (clone D7J2K)	Cell Signaling Technology	12576 (custom formulation*)
Rabbit monoclonal anti-acetyl-Histone H3 (Lys23) (clone RM169)	RevMAb Biosciences	31-1087-00 (custom formulation*)
Mouse monoclonal anti-acetyl-Histone H3 (Lys9) (clone 2G1F9)	Active Motif	61663
Rabbit monoclonal anti-phospho-Histone H2B (Ser14) (clone D67H2)	Cell Signaling Technology	6959 (custom formulation*)
Rabbit monoclonal anti-ubiquityl-Histone H2A (Lys119) (clone D27C4)	Cell Signaling Technology	8240 (custom formulation*)
Rabbit monoclonal anti-acetyl-Histone H3 (Lys18) (clone RM166)	RevMAb Biosciences	31-1055-00 (custom formulation*)
Mouse monoclonal anti-acetyl-Histone H3 (Lys56) (clone 12.1)	Active Motif	61061
Mouse monoclonal anti-peptidylarginine deaminase 4 (PADI4) (clone OTI4H5)	OriGene	CF504813
Rabbit monoclonal anti-ubiquityl-Histone H2B (Lys120) (clone D11)	Cell Signaling Technology	5546 (custom formulation*)
Rabbit monoclonal anti-citrullinated-Histone H3 (Arg2) (clone EPR17703)	abcam	ab176843
Rabbit monoclonal anti-acetyl-Histone H3 (Lys14) (clone D4B9)	Cell Signaling Technology	7627 (custom formulation*)
Rabbit monoclonal anti-acetyl-Histone H4 (Lys16) (clone E2B8W)	Cell Signaling Technology	13534 (custom formulation*)
Mouse monoclonal anti-Histone H4 (clone 31830)	abcam	ab31830
Mouse monoclonal anti-acetyl-Histone H3 (Lys27) (clone MABI 0309)	Active Motif	39685
Mouse monoclonal anti-monomethylarginine (MMA) (clone 5D1)	abcam	ab415
Rabbit monoclonal antisymmetric dimethylarginine (SDMA)	Cell Signaling Technology	13222 (custom formulation*)
Mouse monoclonal anti-dimethyl-Histone H3 (Lys4) (clone MABI 0303)	Active Motif	39679
Mouse monoclonal anti-dimethyl-Histone H3 (Lys9) (clone 5E5-G5)	BioLegend	815501
Mouse monoclonal anti-monomethyl-Histone H3 (Lys9) (clone 7E7.H12)	BioLegend	824201
Rabbit monoclonal anti-trimethyl-Histone H3 (Lys36) (clone RM155)	RevMAb Biosciences	31-1051-00 (custom formulation*)

Mouse monoclonal anti-monomethyl-Histone H3 (Lys27) (clone MABI 0321)	Active Motif	61015
Rabbit monoclonal anti-asymmetric dimethylarginine (ADMA)	Cell Signaling Technology	13522 (custom formulation*)
Mouse monoclonal anti-dimethyl-Histone H3 (Lys36) (clone MABI 0332)	Active Motif	61019
Mouse monoclonal anti-dimethyl-Histone H3 (Lys27) (clone MABI 0324)	Active Motif	61435
Mouse monoclonal anti-dimethyl-Histone H4 (Lys20) (clone MABI 0422)	Active Motif	61533
Rabbit monoclonal anti-Histone H3.3 (clone EPR17899)	abcam	ab176840
Mouse monoclonal anti-trimethyl-Histone H4 (Lys20) (clone 6F8-D9)	BioLegend	827701
Mouse monoclonal anti-macroH2A (clone 14G7)	Millipore	MABE61
Mouse monoclonal anti-trimethyl-Histone H3 (Lys4) (clone G.532.8)	ThermoFisher	MA5-11199 (custom formulation*)
Rabbit monoclonal anti-Histone H2A.Z (clone EPR6171(2)(B))	abcam	ab150402
Rabbit monoclonal anti-monomethyl-Histone H3 (Lys36) (clone EPR16993)	abcam	ab176920
Mouse monoclonal anti-trimethyl-Histone H3 (Lys27) (clone MABI 0323)	Active Motif	61017
Mouse monoclonal anti-monomethyl-Histone H4 (Lys20) (clone 5E10-D8)	BioLegend	828001
Mouse monoclonal anti-CENP-A (clone 3-19)	MBL	D115-3
Mouse monoclonal anti-human CD45- ⁸⁹ Y (clone H130)	Fluidigm	3089003B
Mouse monoclonal anti-human CD4 (clone RPA- T4)	BioLegend	300541
Mouse monoclonal anti-human CD8 (clone SK1)	BioLegend	344727
Mouse monoclonal anti-human CD34 (clone 581)	BioLegend	343531
Mouse monoclonal anti-human CD11c (clone Bu15)	BioLegend	337221
Mouse monoclonal anti-human CD14 (clone M5E2)	BioLegend	301843
Mouse monoclonal anti-human CD33 (clone WM53)	BioLegend	303419
Mouse monoclonal anti-human CD16 (clone B73.1)	BioLegend	360702
Mouse monoclonal anti-human CD123 (clone 9F5)	BD	555642
Mouse monoclonal anti-human CD3 (clone UCHT1)	BioLegend	300443
Mouse monoclonal anti-human CD38 (clone HIT2)	BioLegend	303535
Mouse monoclonal anti-human CD56 (clone NCAM16.2)	BD	559043
Mouse monoclonal anti-human CD19 (clone HIB19)	BioLegend	302247
Mouse monoclonal anti-human HLA-DR (clone L243)	BioLegend	307651

* Custom formulation: PBS, > 1 mg/mL, carrier-free, azide-free