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### **Supplemental Material**

#### **Gestational Perfluoroalkyl Substance Exposure and DNA Methylation at Birth and 12 Years of Age: A Longitudinal Epigenome-Wide Association Study**

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**Additional File-** Excel Document

**Table S1.** Characteristics and maternal serum PFAS concentrations for participants with single or both measures of DNA methylation at birth and/or age 12 years in the HOME Study (Cincinnati, OH; Enrolled 2003-2006).

Characteristics	Both (N= 135) N (%) or Mean (SD)	Single (N= 156) N (%) or Mean (SD)
<b>Child sex</b>		
Girls	71 (53)	83 (53)
Boys	64 (47)	73 (47)
<b>Child age</b>	12.3 (0.7)	12.4 (0.6)
<b>Maternal serum cotinine (ng/mL)</b>		
<0.015 (Unexposed) <sup>a</sup>	37 (28)	59 (38)
0.015-<3 (Secondhand Tobacco Smoke Exposure)	88 (65)	82 (53)
≥3 (Active Smoking)	10 (7)	15 (9)
<b>Maternal race/ethnicity</b>		
White, non-Hispanic	83 (62)	101 (65)
Black, non-Hispanic	45 (33)	43 (28)
All others	7 (5)	12 (7)
<b>Annual household income</b>		
>\$80 K	40 (30)	45 (29)
\$40–80K	47 (35)	50 (32)
\$20–40K	22 (16)	30 (19)
<\$20K	26 (19)	31 (20)
<b>PFAS concentrations (ng/mL)</b>	Median (25 <sup>th</sup> , 75 <sup>th</sup> )	Median (25 <sup>th</sup> , 75 <sup>th</sup> )
PFOA	5.1 (3.6, 7.5)	5.9 (4.2, 8.2)
PFOS	13.3 (9.0, 17.4)	14.3 (10.1, 18.1)
PFHxS	1.2 (0.8, 2.3)	1.7 (1.0, 2.4)
PFNA	0.9 (0.7, 1.2)	1.0 (0.8, 1.3)

<sup>a</sup>Below detection limit. PFAS concentrations were measured in maternal serum at 16 weeks of pregnancy. Note: PFAS, perfluoroalkyl substances; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate. Data were complete for all variables. PFAS concentrations below LOD were replaced with LOD/√2.

**Table S2.** Baseline and follow-up characteristics and maternal plasma PFAS concentrations in the Project Viva (eastern Massachusetts; Enrolled 1999-2002).

Characteristics	Baseline (N=371) N (%)	Follow-up (N=342) N (%)
<b>Child sex</b>		
Female	175 (47)	163 (48)
Male	196 (53)	179 (52)
<b>Child race</b>		
White	263 (71)	229 (67)
Non-white	108 (29)	113 (33)
<b>Annual household income</b>		
>\$70,000	222 (60)	210 (62)
≤\$70,000	149 (40)	132 (38)
<b>Maternal smoking status</b>		
Smoked in pregnancy	42 (11)	39 (11)
Did not smoke in pregnancy	329 (89)	303 (89)
<b>PFAS concentrations (ng/mL)</b>		
	<b>Median (25<sup>th</sup>, 75<sup>th</sup>)</b>	<b>Median (25<sup>th</sup>, 75<sup>th</sup>)</b>
PFOA	5.7 (4.1, 7.5)	5.2 (3.6, 7.2)
PFOS	25.6 (18.5, 34.5)	23.2 (16.9, 32.4)
PFHxS	2.3 (1.6, 3.8)	2.2 (1.5, 3.7)
PFNA	0.7 (0.5, 0.9)	0.6 (0.5, 0.9)

Note: PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; mean and SD for child age at follow-up visit was 7.9 (0.8) years. No missing values (excluded in participant flow if missing exposure, outcome, covariate).

**Table S3.** Distribution of serum per- and polyfluoroalkyl substance (PFAS) concentrations (ng/mL) in pregnancy (n=291) in the HOME Study (Cincinnati, OH; Enrolled 2003-2006)

<b>PFAS</b>	<b>Minimum</b>	<b>25<sup>th</sup> Pctl</b>	<b>50<sup>th</sup> Pctl</b>	<b>75<sup>th</sup> Pctl</b>	<b>Maximum</b>
PFHxS	0.1	0.9	1.5	2.3	32.5
PFNA	0.3	0.7	0.9	1.2	2.9
PFOA	1.1	3.8	5.4	7.8	26.4
PFOS	1.4	9.6	13.7	18.0	57.2

**Table S4.** Pearson correlation between log<sub>2</sub>-transformed serum per- and polyfluoroalkyl substance (PFAS) concentrations (ng/mL) in pregnancy (n=291) in the HOME study (Cincinnati, OH; Enrolled 2003-2006).

	<b>PFOA</b>	<b>PFOS</b>	<b>PFNA</b>	<b>PFHxS</b>
<b>PFOA</b>	1.00	0.58	0.45	0.44
<b>PFOS</b>		1.00	0.50	0.63
<b>PFNA</b>			1.00	0.29
<b>PFHxS</b>				1.00

**Table S5.** Genes from statistically significant PFAS associated CpG sites and their potential human health implications in the HOME Study (Cincinnati, OH; Enrolled 2003-2006).

Gene	Description	PFAS	Phenotype
MAGI1	Membrane Associated Guanylate Kinase, WW and PDZ Domain Containing 1	PFOA	Breast cancer
KRT18	Keratin 18	PFOA	Prostate cancer; breast cancer-associated fibroblasts
SRPRB	Signal Recognition Particle Receptor Subunit Beta	PFOA	Pancreatic ductal adenocarcinoma
TNR	Tenascin R	PFOA	Neural development; brain cancer
SLC10A2	Solute Carrier Family 10 Member 2	PFOA	Alzheimer's disease; cholesterol metabolism
LOC102724050	Uncharacterized LOC102724050	PFOA	BMI
AGAP1	ArfGAP With GTPase Domain, Ankyrin Repeat and PH Domain 1	PFOA	Obesity; coronary artery disease
SERPINA5	Serpin Family A Member 5	PFOA	Kidney function; reproductive system; prostate cancers;
HPSE2	Heparanase 2 (Inactive)	PFOS	Breast cancer; type II diabetes mellitus
HIF1A	Hypoxia Inducible Factor 1 Subunit Alpha	PFNA	Pancreatic cancer; prostate cancer
C14orf101	Chromosome 14 Open Reading Frame 101	PFNA	Gastric cancer; non-Hodgkin lymphoma
RADIL	Rap Associating with DIL Domain	PFNA	Lung adenocarcinoma
DPAGT1	Dolichyl-Phosphate N-Acetylglucosaminophosphotransferase 1	PFNA	Neuromuscular transmission disorders
SLC6A2	Solute Carrier Family 6 Member 2	PFNA	ADHD; depression; bipolar disorder
TMEM56	Transmembrane Protein 56	PFNA	Neurodevelopmental disorders; bipolar disorder
RNF13	Ring Finger Protein 13	PFNA	Coronary artery disease; Parkinson's disease; pancreatic cancers
EPB41L3	Erythrocyte Membrane Protein Band 4.1 Like 3	PFNA	Kidney function; prostate cancer; gastric cancer;
VIPR1	Vasoactive Intestinal Peptide Receptor 1	PFHxS	Inflammatory bowel disease; lung adenocarcinoma; hepatocellular carcinoma;
CDSN; PSORS1C1	Corneodesmosin; Psoriasis Susceptibility 1 Candidate 1	PFHxS	Psoriasis susceptibility; rheumatoid arthritis

**Table S6.** A list of CpGs with p-value  $\leq 0.001$  provided by prior studies with the same direction of association and p-value  $< 0.05$  observed in the HOME Study (Cincinnati, OH; Enrolled 2003-2006)

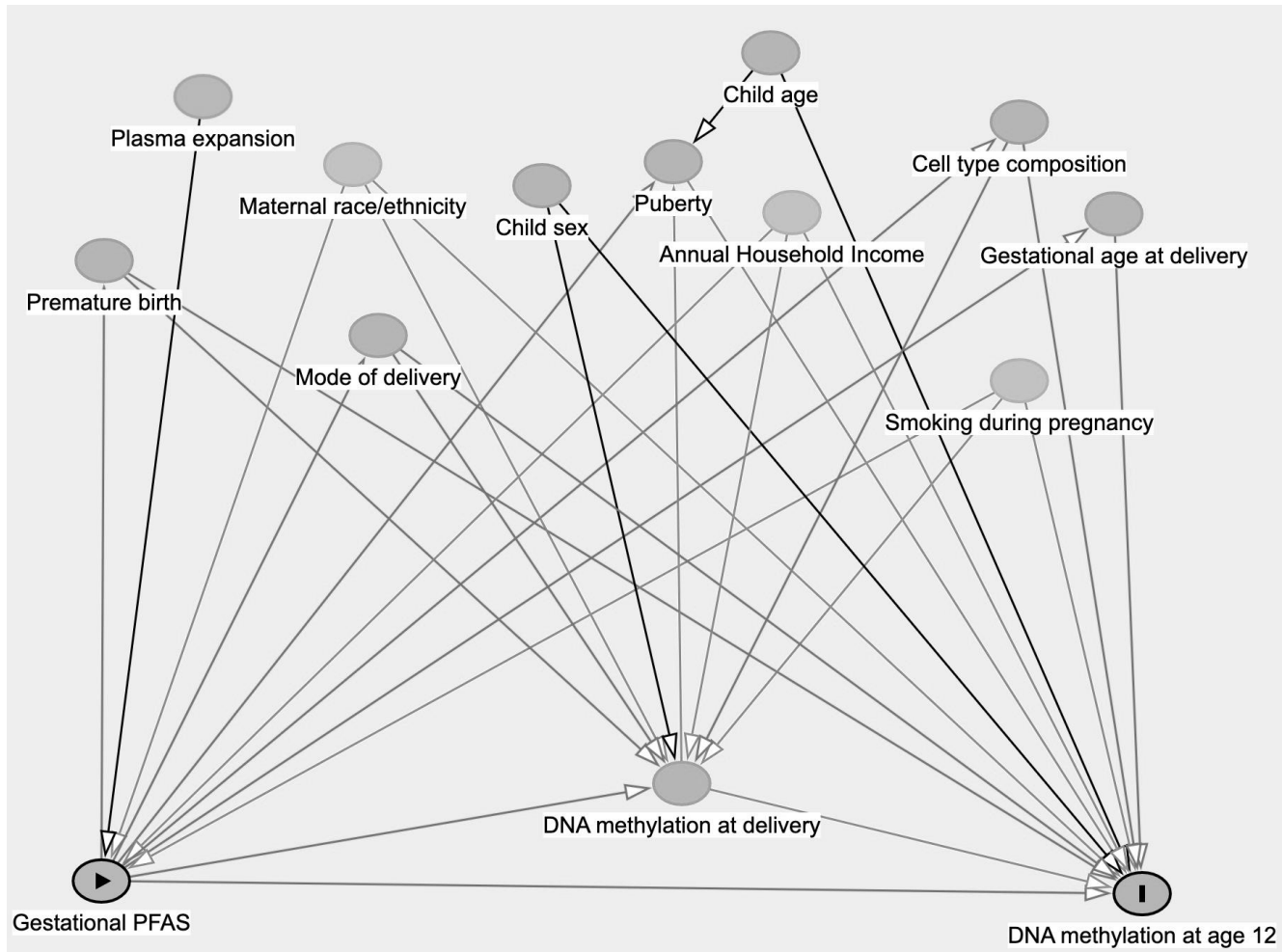
CpG	Chr	CpG Location	Results from Prior Studies					Results from HOME Study			
			Gene	Gene Region	$\beta$	P-value	PFAS	Study	$\beta$	P-value	
cg00057676	1	island	C1orf220	Body	-0.003	6.33E-04	PFOA	Miura et al.	-0.064	0.021	
cg03124880	12	open sea	WSCD2	Body	0.024	2.99E-04	PFOA	Miura et al.	0.071	0.009	
cg03173827	20	island	TCEA2	1stExon	-0.024	6.77E-04	PFOA	Miura et al.	-0.021	0.032	
cg03191504	2	shore	RNF149	Body	-0.013	8.58E-04	PFOA	Miura et al.	-0.048	0.031	
cg05012972	7	island	PTPRN2	Body	-0.037	2.51E-04	PFOA	Miura et al.	-0.053	0.017	
cg11897736	13	shore	MCF2L	Body	0.011	2.36E-04	PFOA	Miura et al.	0.058	0.024	
cg15224291	18	open sea	MBP	TSS200	0.007	4.73E-04	PFOA	Miura et al.	0.052	0.022	
cg15997374	11	shelf	ATG2A	Body	0.002	5.73E-04	PFOA	Miura et al.	0.025	0.032	
cg16022876	11	shore	BRSK2	Body	0.009	3.84E-04	PFOA	Miura et al.	0.051	0.007	
cg16214582	4	island	RP3-513G18.2	IGR	0.019	8.96E-04	PFOA	Miura et al.	0.051	0.024	
cg16362594	6	shore	C6orf123	IGR	0.006	4.86E-04	PFOA	Miura et al.	0.058	0.047	
cg15272467	20	island	YTHDF1	IGR	0.035	6.21E-04	PFOS	Miura et al.	0.060	0.000	
cg14426688	7	island	STK17A	Body	0.006	9.11E-05	PFOS	Miura et al.	0.032	0.015	
cg09475757	4	island	NEIL3	TSS200	0.018	8.90E-04	PFOS	Miura et al.	0.052	0.036	
cg05146852	11	open sea	ANO3	Body	0.031	1.78E-04	PFOS	Miura et al.	0.077	0.005	
cg26954114	15	open sea	MIR1469	IGR	0.072	4.87E-04	PFOS	Miura et al.	0.040	0.008	
cg11835209	5	open sea	LINC01020	IGR	0.042	7.70E-04	PFOS	Miura et al.	0.043	0.010	
cg14788655	11	open sea	LRRC4C	5'UTR	0.041	2.99E-05	PFOS	Miura et al.	0.069	0.012	
cg06899970	8	open sea	DKK4	5'UTR	0.012	7.81E-04	PFOS	Miura et al.	0.065	0.017	
cg00583535	1	open sea	IGFN1	TSS200	0.041	5.85E-04	PFOS	Miura et al.	0.052	0.019	
cg09928274	19	open sea	ELSPBP1	5'UTR	0.022	8.72E-06	PFOS	Miura et al.	0.062	0.023	
cg09618309	2	open sea	LYG1	Body	0.01	1.46E-04	PFOS	Miura et al.	0.052	0.031	
cg09860486	3	open sea	IQCF4	IGR	0.026	5.00E-04	PFOS	Miura et al.	0.044	0.035	
cg27593250	6	open sea	DDR1	Body	0.035	6.18E-04	PFOS	Miura et al.	0.035	0.044	
cg04057106	9	open sea	KIF24	Body	0.028	4.63E-04	PFOS	Miura et al.	0.076	0.049	
cg14321373	8	shelf	KCNK9	3'UTR	0.008	7.37E-04	PFOS	Miura et al.	0.131	0.002	
cg25122824	7	shore	MAD1L1	Body	-0.019	7.90E-04	PFOS	Miura et al.	-0.104	0.003	
cg24218620	17	shore	DHRS13	Body	0.039	5.57E-04	PFOS	Miura et al.	0.041	0.005	
cg25697152	12	shore	TEAD4	TSS1500	0.023	5.17E-05	PFOS	Miura et al.	0.064	0.006	
cg00220225	3	shore	SEMA3F	5'UTR	0.043	5.56E-04	PFOS	Miura et al.	0.045	0.009	
cg18486150	1	shore	KIF17	TSS1500	0.025	2.02E-04	PFOS	Miura et al.	0.049	0.011	
cg07455279	19	shore	NDUFA3	TSS1500	0.044	2.83E-05	PFOS	Miura et al.	0.077	0.013	
cg05162166	22	shore	ZC3H7B	IGR	0.019	9.03E-04	PFOS	Miura et al.	0.023	0.018	
cg20251943	10	shore	DIP2C	Body	0.069	9.95E-05	PFOS	Miura et al.	0.047	0.039	
cg18155888	1	shore	MORN1	Body	0.017	4.32E-06	PFOS	Miura et al.	0.093	0.043	
cg00890632	10	OpenSea	PFKFB3	Body	0.027	0.0008	PFOS	Starling et al.	0.047	0.039	
cg01253508	5	Island	-	-	-0.020	0.0005	PFOS	Starling et al.	-0.053	0.036	
cg01861377	19	OpenSea	PPAP2C	Body	0.026	0.0002	PFOS	Starling et al.	0.024	0.037	
cg01909551	3	OpenSea	-	-	-0.030	0.0007	PFOS	Starling et al.	-0.041	0.003	



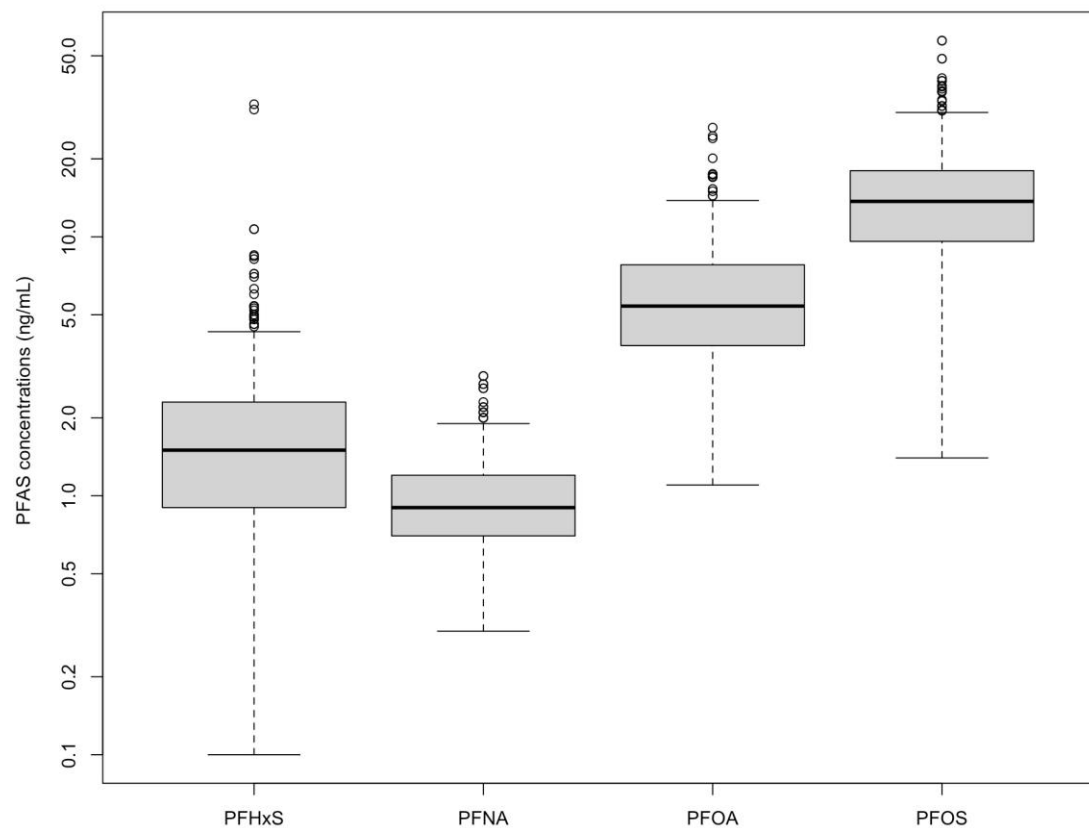
cg03950246	15	N_Shore	NEO1	TSS1500	0.049	0.0002	PFOS	Starling et al.	0.046	0.029
cg14827285	13	N_Shelf	-	-	-0.039	0.0008	PFOS	Starling et al.	-0.059	0.010
cg16476639	14	S_Shelf	RBM25	5'UTR	-0.038	0.001	PFOS	Starling et al.	-0.050	0.038
cg23987134	5	OpenSea	LOC285696	Body	0.045	0.0009	PFOS	Starling et al.	0.034	0.017
cg00244391	6	S_Shelf	C6orf182	5'UTR	-0.035	1.96E-04	PFNA	Starling et al.	-0.074	0.021
cg09706988	11	Island	AQP11	TSS200	-0.044	2.38E-04	PFNA	Starling et al.	-0.064	0.008
cg11299207	6	N_Shore	EHMT2	TSS1500	0.029	4.81E-04	PFNA	Starling et al.	0.046	0.045
cg19346084	22	Island	-	-	0.119	6.15E-04	PFNA	Starling et al.	0.146	0.041
cg19363466	19	Island	MZF1; LOC100131691	Body	0.042	8.17E-04	PFNA	Starling et al.	0.068	0.020
cg21330423	6	Island	HLA-J;NCRNA00171	Body	-0.063	5.23E-05	PFNA	Starling et al.	-0.094	0.002
cg22251017	19	Island	APLP1	Body	0.0147	5.52E-04	PFNA	Starling et al.	0.051	0.044
cg26831259	4	S_Shore	KIAA1530	Body	0.0277	6.40E-04	PFNA	Starling et al.	0.089	0.004
cg27228559	10	Island	CASC2;RAB11FIP2	Body;TSS1500	-0.032	8.75E-04	PFNA	Starling et al.	-0.073	0.023
cg00039070	7	OpenSea	EXOC4	Body;Body	0.039	1.91E-04	PFHxS	Starling et al.	0.038	0.045
cg07052513	2	Island	KDM3A	5'UTR;TSS200;1stExon	0.033	8.70E-04	PFHxS	Starling et al.	0.029	0.017
cg08015507	3	N_Shelf	NCKIPSD	Body	0.013	9.00E-04	PFHxS	Starling et al.	0.023	0.049
cg10692932	12	Island	TROAP	TSS200	-0.052	2.27E-04	PFHxS	Starling et al.	-0.027	0.014
cg14328535	2	OpenSea	MYT1L	Body	0.0315	2.32E-04	PFHxS	Starling et al.	0.046	0.008
cg16433800	2	Island	PDK1	1stExon;5'UTR	-0.053	4.47E-04	PFHxS	Starling et al.	-0.015	0.032
cg22661082	2	OpenSea	-	-	0.030	3.71E-04	PFHxS	Starling et al.	0.047	0.005
cg00330059	4	-	ODZ3	Body	0.64	0.01	PFOS	Xu et al.*	0.077	0.014
cg01192291	6	S_Shore	ZKSCAN4	TSS1500	0.7	0.003	PFOS	Xu et al.	0.070	0.013
cg09157632	15	-	PIGB	Body	0.73	0.013	PFOS	Xu et al.	0.062	0.019
cg09840146	5	-	-	-	0.12	0.005	PFOS	Xu et al.	0.140	0.001
cg16694239	1	-	PEA15	5'UTR;Body;TSS1500	0.61	0.003	PFOS	Xu et al.	0.031	0.007
cg25025310	1	-	-	-	0.84	0.013	PFOS	Xu et al.	0.090	0.005

Note: PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; -, not annotated to any known gene; NA, not available in 450K microarray; Chr, chromosome; Body, between the ATG and stop codon; 5'UTR, between the TSS and the ATG start site; 1stExon, the first exon of the gene; TSS, transcription start site; TSS1500, 1500 bases from TSS; FDR, false discovery rate. Statistically significant CpG sites were defined as having FDR q-value <0.05 (PFOA, PFOS, and PFHxS) or 0.01 (PFNA); CpG sites were annotated to a promoter region if they were assigned to TSS200 or TSS1500. All models were adjusted for child age and sex, annual household income, maternal smoking during pregnancy and race, and cell type composition. \*Only q-values are available for Xu's study. CpGs with the same direction of effect and a P-value < 0.05 are shown.

**Figure S1.** Directed acyclic graph of potential confounders of the associations between serum PFAS concentrations in pregnancy and DNA methylation at birth and age 12 years in the HOME Study (Cincinnati, OH; Enrolled 2003-2006)

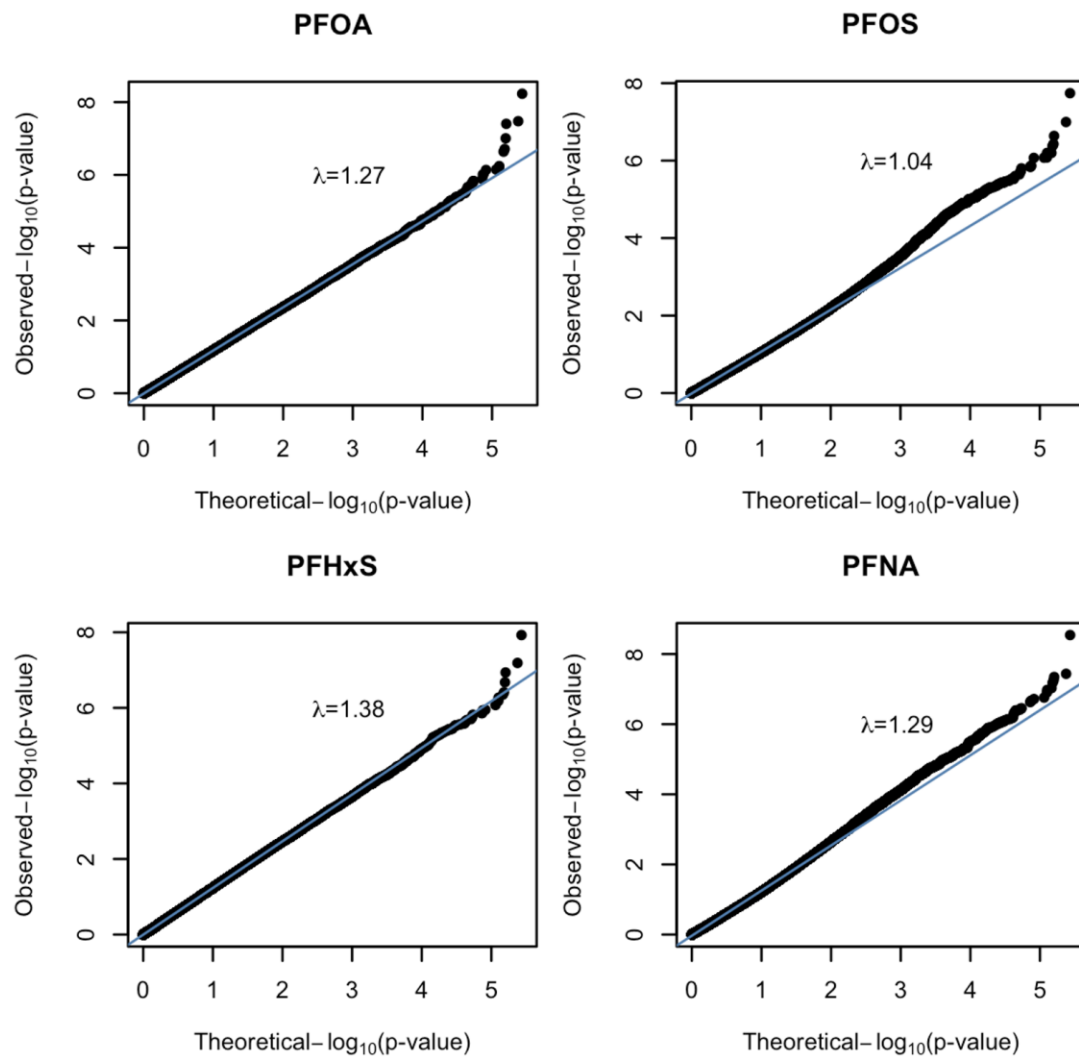


**Figure S2.** Box plot of serum per- and polyfluoroalkyl substance (PFAS) concentrations (ng/mL) in pregnancy in the HOME Study (Cincinnati, OH; Enrolled 2003-2006)



Note: PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; FDR, false discovery rate; Dots placed past the line edges indicate outliers.

**Figure S3.** Quantile-quantile plots for the associations between gestational PFAS concentrations and repeated measures of DNA methylation in the HOME Study (Cincinnati, OH; Enrolled 2003-2006)



Note: PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate. Adjusted for child age and sex, annual household income, maternal smoking during pregnancy and race, and cell type composition.