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Supplemental Material

DNA Methylation Score as a Biomarker in Newborns for Sustained Maternal Smoking during Pregnancy

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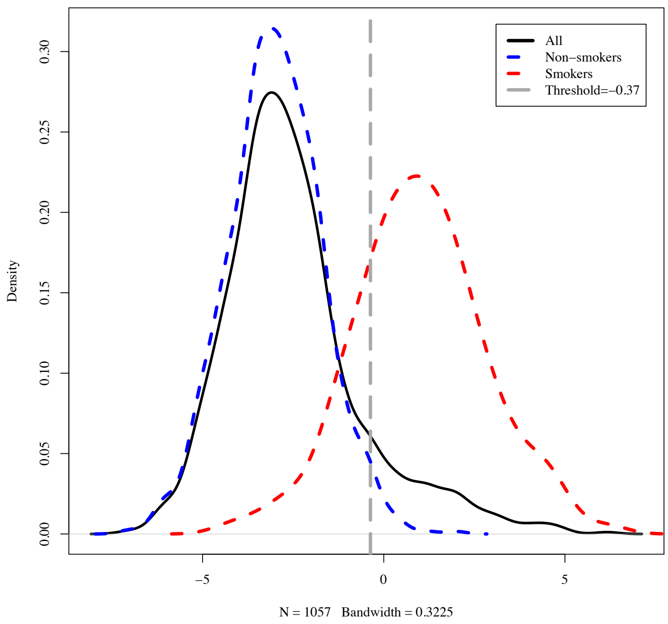
A: Training Set



B: Test Set

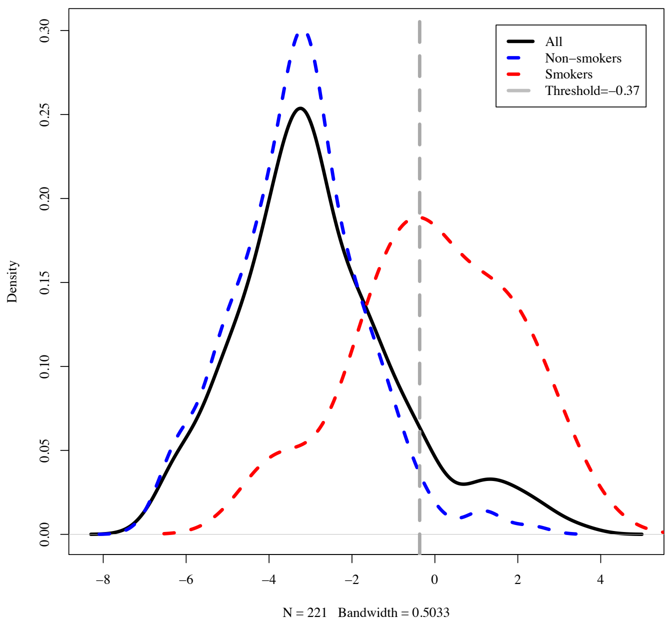
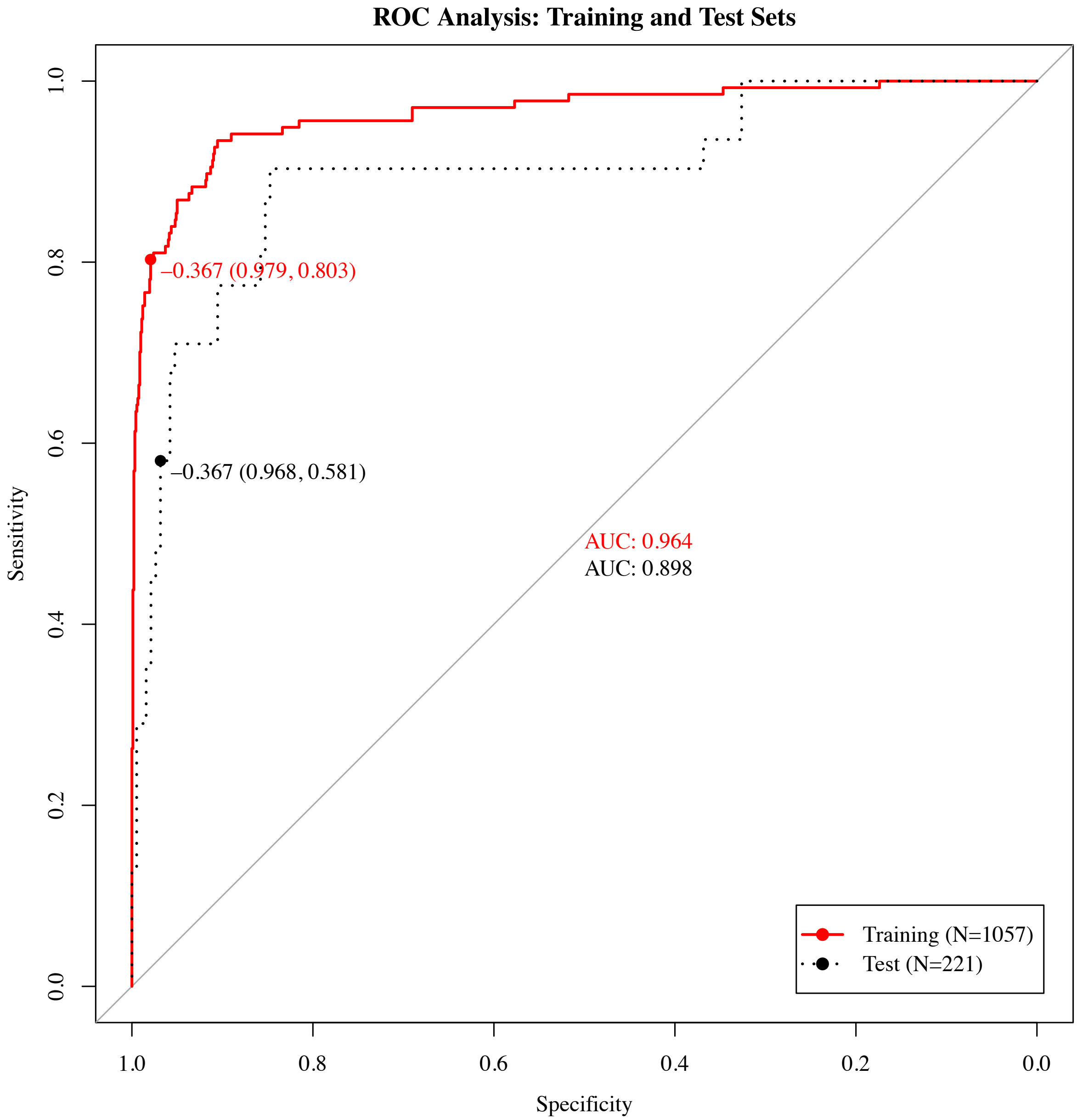


Figure S2: Plot showing the combined sustained smoking model ROC curves for the training (red solid line) and test (black dotted line) datasets. Cutoff score of -0.37 developed in the training set is shown on both curves, along with the corresponding specificity and sensitivity.



Supplementary Tables

Table S1: 28 CpGs selected by the logistic LASSO regression model using combined sustained smoking*.

CpG	Coefficient	Gene Name
cg00709966	-1.667	
cg02256631	-0.191	*ITGAM*
cg02482603	2.706	*RABGAP1L*
cg04103532	1.786	*HIVEP2*
cg04180046	14.027	*MYO1G*
cg04506190	2.318	*PLXND1*
cg05549655	6.210	*CYP1A1*
cg05575921	-10.909	*AHRR*
cg08698721	1.142	*MEG3*
cg09743950	-6.330	*ITGAM*
cg10799846	-4.963	*SYNJ2*
cg11864574	-0.370	*SPAG6*
cg12186702	3.847	*PLVAP*
cg13834112	1.514	
cg13893782	-0.963	*PDE7B*
cg14179389	-6.304	*GFI1*
cg14351425	6.361	*GRK5*
cg14633298	5.050	*TRIM27*
cg14743346	2.286	*DERL1*
cg17397069	-2.912	*SGCD*
cg19381766	5.245	
cg22154659	-0.773	*HOXA1*
cg22802102	-0.254	*NTF3*
cg23304605	0.011	*CCDC88C*
cg25189904	-3.903	*GNG12*
cg25949550	-46.991	*CNTNAP2*
cg26764244	-0.246	*GNG12*
cg27291468	0.836	

* In the combined sustained smoking variable, a woman's positive report of daily smoking during pregnancy overrides a cotinine value of ≤56.8 nmol/L used to classify a woman as a non-smoker in the cotinine-based sustained smoking variable.

Table S2: Cross-tabulation of cotinine-based and self-reported sustained smoking

Cotinine-based Sustained Smoking	**Self-reported Sustained Smoking**	**Training (MoBa1; n (%))**	**Test (MoBa2; n (%))**
Non-smoker	Non-smoker	910 (86.1)	186 (84.2)
Non-smoker	Smoker	20 (1.9)	5 (2.3)
Smoker	Non-smoker	26 (2.5)	5 (2.3)
Smoker	Smoker	101 (9.5)	25 (11.3)

Table S3: 24 CpGs selected by the logistic LASSO regression model using cotinine-based sustained smoking.

CpG	Coefficient	Gene Name
cg00044354	-0.657	*MIER1;WDR78*
cg00709966	-1.145	
cg04180046	12.144	*MYO1G*
cg04506190	2.072	*PLXND1*
cg05371791	-2.670	*ANKMY1*
cg05549655	5.353	*CYP1A1*
cg05575921	-12.766	*AHRR*
cg06686709	6.381	*CACNB4*
cg07339236	-0.052	*ATP9A*
cg08872493	-0.139	*HTR1D*
cg09743950	-3.092	*ITGAM*
cg09935388	-3.588	*GFI1*
cg12803068	0.122	*MYO1G*
cg12806681	-0.464	*AHRR*
cg13834112	2.253	
cg14179389	-6.339	*GFI1*
cg14351425	5.049	*GRK5*
cg19381766	4.924	
cg20294319	3.181	*LRCH1*
cg22154659	-2.932	*HOXA1*
cg22875872	4.801	*PTPRF*
cg25189904	-2.030	*GNG12*
cg25949550	-38.434	*CNTNAP2*
cg26764244	-1.471	*GNG12*

Table S4: 12 CpGs selected by the logistic LASSO regression model using self-reported sustained smoking

CpG	**Coefficient**	**Gene Name**
cg00253658	1.361	
cg04180046	8.403	*MYO1G*
cg05549655	3.467	*CYP1A1*
cg05575921	-11.139	*AHRR*
cg09743950	-1.710	*ITGAM*
cg09935388	-2.675	*GFI1*
cg11429111	0.125	
cg13787193	-13.339	*LOC148709*
cg14179389	-1.518	*GFI1*
cg17681313	-4.307	*CYP20A1*
cg23304605	0.681	*CCDC88C*
cg25949550	-38.772	*CNTNAP2*

Table S5: 26 CpGs selected by the logistic LASSO regression model using combined sustained smoking restricted to CpGs that overlap with the IlluminaEPIC BeadChip.

CpG	**Coefficient**	**Gene Name**
cg02256631	-0.191	*ITGAM*
cg02482603	2.706	*RABGAP1L*
cg04103532	1.786	*HIVEP2*
cg04180046	14.027	*MYO1G*
cg04506190	2.318	*PLXND1*
cg05549655	6.210	*CYP1A1*
cg05575921	-10.909	*AHRR*
cg08698721	1.142	*MEG3*
cg09743950	-6.330	*ITGAM*
cg10799846	-4.963	*SYNJ2*
cg12186702	3.847	*PLVAP*
cg13834112	1.514	
cg13893782	-0.963	*PDE7B*
cg14179389	-6.304	*GFI1*
cg14351425	6.361	*GRK5*
cg14633298	5.050	*TRIM27*
cg14743346	2.286	*DERL1*
cg17397069	-2.912	*SGCD*
cg19381766	5.245	
cg22154659	-0.773	*HOXA1*
cg22802102	-0.254	*NTF3*
cg23304605	0.011	*CCDC88C*
cg25189904	-3.903	*GNG12*
cg25949550	-46.991	*CNTNAP2*
cg26764244	-0.246	*GNG12*
cg27291468	0.836	

Table S6: Logistic LASSO results for main model (combined sustained smoking), the main model using log ratios, and the main model using only the CpGs available on the IlluminaEPIC array– The number of CpGs (*q*) used to calculate the smoking methylation score, area under the curve (AUC) and 95% confidence interval (CI), smoking methylation score threshold, percent accuracy and CI, sensitivity and CI, specificity and CI, and the number and percentage of false negatives (FN) and false positives (FP).

Model		***q***	**AUC (CI)**	**Threshold**	**Accuracy (CI)**	**Sensitivity (CI)**	**Specificity (CI)**	**FN** (%)	**FP** (%)
Combined Sustained Smoking	Training	28	0.96 (0.95,0.98)	-0.37	0.96 (0.94,0.97)	0.80 (0.74,0.87)	0.98 (0.97,0.99)	27 (2.6)	19 (1.8)
	Test		0.90 (0.83,0.97)		0.91 (0.88,0.95)	0.58 (0.39,0.74)	0.97 (0.94,0.99)	13 (5.9)	6 (2.7)
Combined Sustained Smoking – log ratios	Training	37	0.97 (0.95,0.99)	7.41	0.95 (0.94,0.96)	0.82 (0.76,0.88)	0.97 (0.96,0.98)	24 (2.2)	27 (2.6)
	Test		0.90 (0.82,0.97)		0.91 (0.88,0.95)	0.58 (0.39,0.74)	0.97 (0.94,0.99)	13 (5.9)	6 (2.7)
Combined Sustained Smoking – EPIC	Training	26	0.96 (0.95,0.98)	0.33	0.95 (0.94,0.96)	0.81 (0.75,0.88)	0.97 (0.96,0.98)	26 (2.5)	27 (2.6)
	Test		0.90 (0.83,0.97)		0.91 (0.88,0.95)	0.65 (0.45,0.81)	0.96 (0.93,0.98)	11 (5.0)	8 (3.6)

Table S7: Descriptive statistics of smoking variables in the training data set*.

Variable		**Training**
Methylation Score Class (n (%))	No	907 (87.3)
	Yes	132 (12.7)
Cotinine-based Sustained Smoking (n (%))	No	912 (87.8)
	Yes	127 (12.2)
Self-reported Sustained Smoking (n (%))	No	918 (88.4)
	Yes	121 (11.6)
Combined Sustained Smoking (n (%))	No	902 (86.8)
	Yes	137 (13.2)
Self-reported Any Smoking (n (%))	No	745 (71.7)
	Yes	294 (28.3)

* Note: Participants with nonmissing data for all covariates were used for the birth weight analyses; therefore, the sample counts in this table are slightly different for the smoking variables between the main and sensitivity analyses (N=1,057; Table 1) compared with the birth weight analyses (N=1,039).

Table S8: Descriptive statistics of birth weight and maternal and newborn characteristics in the training data set.

Variable	Training
N*	1039
Birth Weight (g) (mean ± sd)	3644 ± 543.6
Gestational Age (weeks) (mean ± sd)	40.0 ± 1.6
Mother's Age (years) (mean ± sd)	29.9 ± 4.3
Sex - Male (n (%))	550 (52.9)
Parity ≥ 1 (n (%))	617 (59.4)
Maternal Education	
<High School (n (%))	78 (7.5)
High School (n (%))	334 (32.1)
Some College (n (%))	460 (44.3)
+4yr College (n (%))	167 (16.1)

* Note: Participants with nonmissing data for all covariates were used for the birth weight analyses; therefore, the total sample size was slightly lower for the birth weight analyses (N=1,039).

Table S9: Mean Methylation Values of the 28 CpGs selected by the main model (combined sustained smoking).

CpG	Mean
cg00709966	0.4105
cg02256631	0.1028
cg02482603	0.4274
cg04103532	0.5712
cg04180046	0.4513
cg04506190	0.2577
cg05549655	0.1966
cg05575921	0.8649
cg08698721	0.6021
cg09743950	0.6816
cg10799846	0.2261
cg11864574	0.4560
cg12186702	0.5048
cg13834112	0.5733
cg13893782	0.1812
cg14179389	0.2388
cg14351425	0.2197
cg14633298	0.8567
cg14743346	0.7659
cg17397069	0.6746
cg19381766	0.5556
cg22154659	0.4234
cg22802102	0.7330
cg23304605	0.1827
cg25189904	0.5317
cg25949550	0.1111
cg26764244	0.2733
cg27291468	0.6715

Table S10: 37 CpGs selected by the logistic LASSO regression model on the log ratios using combined sustained smoking.

CpG	**Coefficient**	**Gene Name**
cg00044354	-0.036	*MIER1;WDR78*
cg00709966	-0.476	
cg02256631	-0.108	*ITGAM*
cg02482603	0.906	*RABGAP1L*
cg02973307	-0.015	*KCTD15*
cg03991871	-0.639	*AHRR*
cg04103532	0.518	*HIVEP2*
cg04180046	3.000	*MYO1G*
cg04506190	0.596	*PLXND1*
cg05549655	1.022	*CYP1A1*
cg05575921	-1.328	*AHRR*
cg05757757	0.103	
cg05854217	-0.093	
cg08698721	0.234	*MEG3*
cg09273054	-0.021	*MAL2*
cg09743950	-1.508	*ITGAM*
cg10673740	0.180	*BAG3*
cg10799846	-0.969	*SYNJ2*
cg11348106	0.085	*SEC14L1*
cg11864574	-0.283	*SPAG6*
cg12186702	1.254	*PLVAP*
cg13834112	0.846	
cg13893782	-0.164	*PDE7B*
cg14179389	-1.220	*GFI1*
cg14351425	1.214	*GRK5*
cg14633298	0.855	*TRIM27*
cg14743346	0.339	*DERL1*
cg17397069	-0.706	*SGCD*
cg18422587	0.187	
cg19381766	1.214	
cg22154659	-0.277	*HOXA1*
cg23304605	0.125	*CCDC88C*
cg25189904	-1.113	*GNG12*
cg25949550	-4.500	*CNTNAP2*
cg26038465	0.111	*MAFF*
cg26709433	0.012	
cg27291468	0.195	