



## **Supplemental Material to:**

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**Postnatal stability and tissue- and time-specific effects of AHRR methylation change in response to maternal smoking throughout pregnancy**

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**<http://www.landesbioscience.com/admin/article/27248/>**

**Supplementary figure 1. Map of the *AHRR* region of interest.** A) *AHRR* gene showing exons and introns, CGIs (green rectangles), Infinium HM450 probes (blue vertical lines), smoking-associated probes (vertical lines of various colours, matched to original study), and the active histone mark (H3K4Ac) UCSC track. B) close up view of the *AHRR* region of interest, showing the three EpiTYPER assays (A, B and C) and the CGI (green rectangle). C) MassARRAY cleavage pattern for each assay, showing analysable CpG sites. In total 32 CpG sites, contained within 18 CpG units (white circles with black outline), were available for analysis.

**Supplementary figure 2. There was no association between maternal smoking and *AHRR* methylation in buccal epithelium and placenta.** No detectable differences in methylation were observed at any CpG site between the smoked throughout and never smoked groups in buccals and placenta. This suggests that maternal smoking effects on DNA methylation are restricted to CBMCs. X axis = CpG unit, y axis = DNA methylation.

**Supplementary figure 3. There was no association between sex and *AHRR* methylation.** Individuals were separated by sex, in order to determine if smoking associated change in *AHRR* methylation was more pronounced in males or females. x axis (group and sex), y axis – DNA methylation.

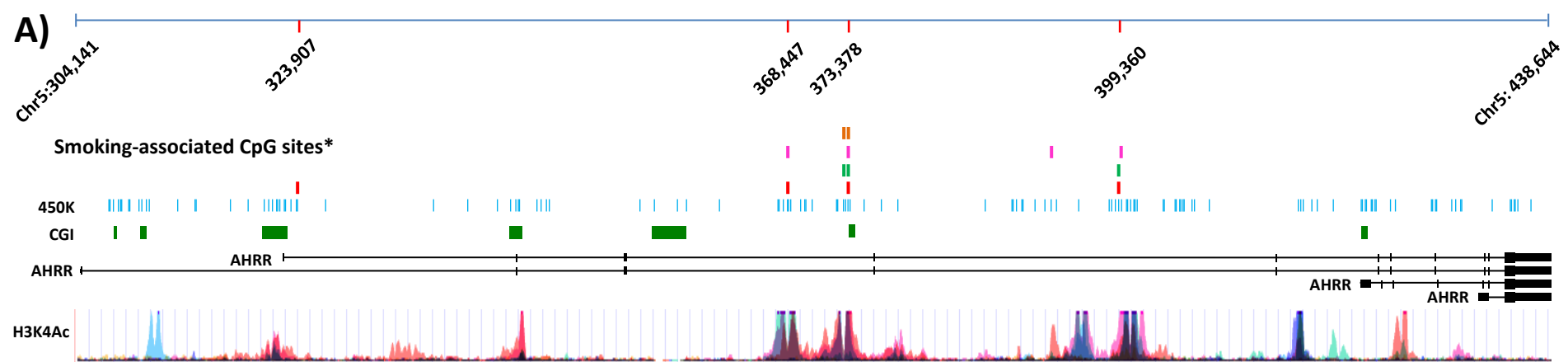
**Supplementary figure 4. No association between maternal smoking and *GFI1* methylation in CBMCs.** In order to test the reproducibility of *GFI1* hypomethylation in response to maternal smoking, reported by Joubert et al. (2012), we targeted the region of the gene that showed the largest change in methylation in that study. There was no association between maternal smoking and methylation across all 24 CpG sites, within 12 CpG units. Error bars = 95% CI.

**Supplementary figure 5. *MYO1G* methylation in CBMCs exposed to maternal smoking.**

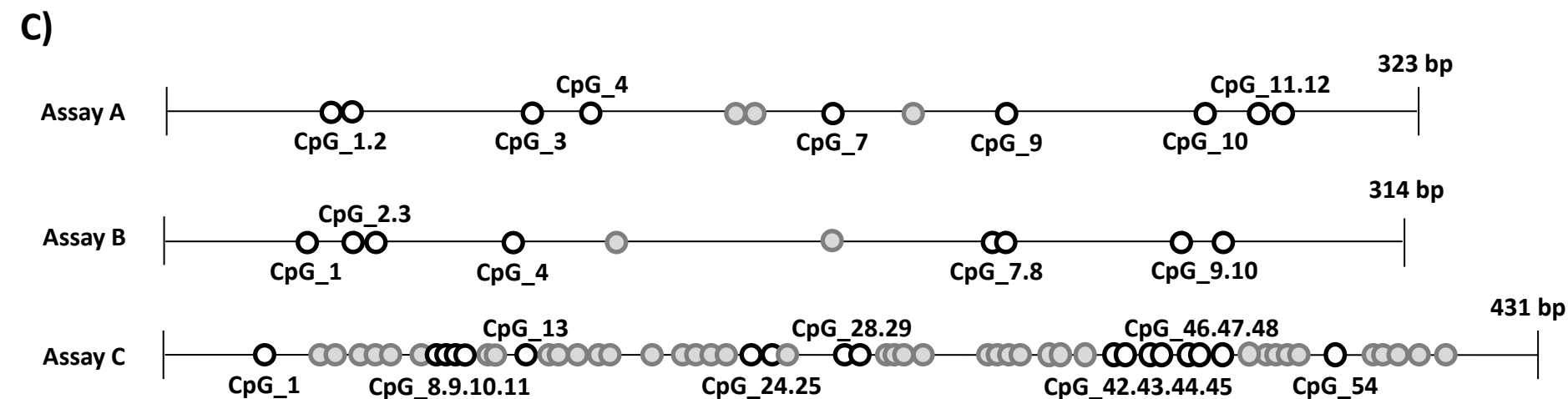
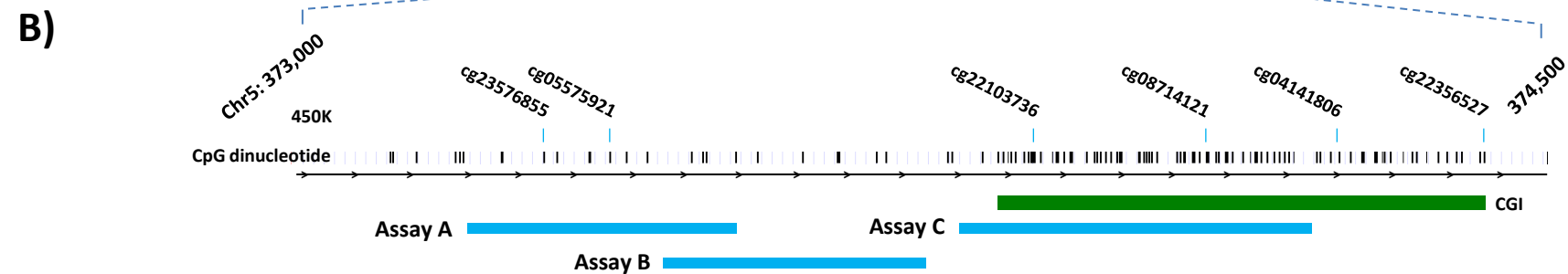
*MYO1G* methylation was quantified using an EpiTYPER assay that targeted 2 CpG sites identified by Joubert et al. (2012) to be associated with maternal smoking. Our analysis suggests a trend towards higher *MYO1G* methylation in the smoking exposed group, however, due to samples size the p value remained greater than 0.05. Error bars = 95% CI

**Supplementary figure 6. *AHRR* methylation in individual 18 month mononuclear cell samples.** X axis shows individual CpG sites across assay A and B, and y axis shows DNA methylation level. Individuals belonging to the ‘Smoked throughout’ group are shown as brown dashed lines and circles, individuals in the ‘never smoked’ group are shown as black lines and circles. Generally all individuals in the smoked throughout group, showed lower methylation than individuals in the never smoked group.

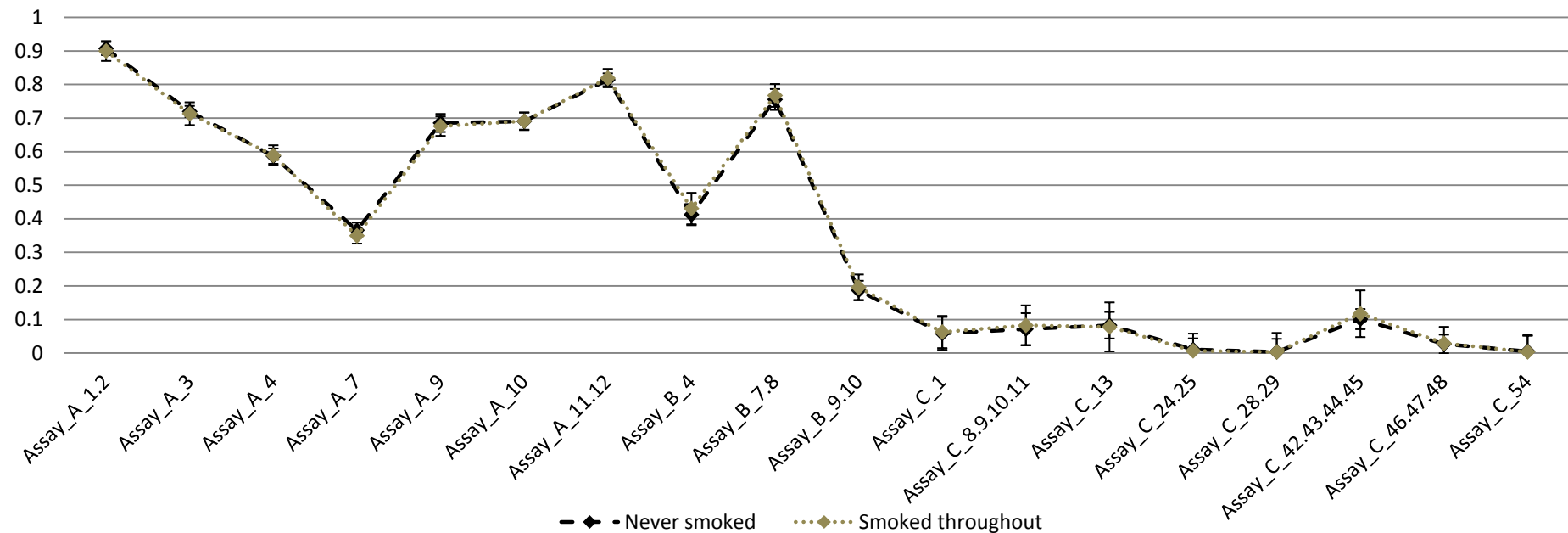
**Supplementary figure 7. Mean difference in methylation between the smoking and controls groups at birth and 18 months.** The mean difference in methylation at 18 months shows the same direction as the birth samples (hypomethylation in smoked throughout group), however the absolute difference in methylation is lower at 18 months. X axis – individual CpG units, y axis – difference in methylation (dB) between smoked throughout and never smoked groups.



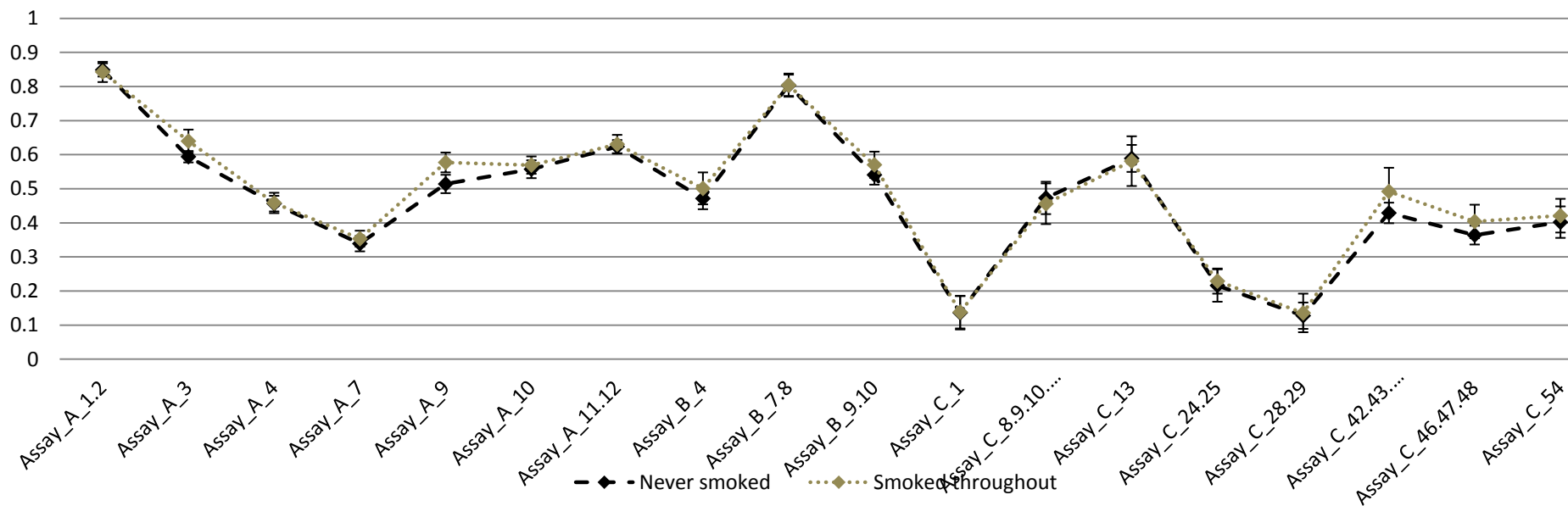
\* | Joubert, 2012 | Shenker, 2012 | Monick, 2012 | Philibert, 2012



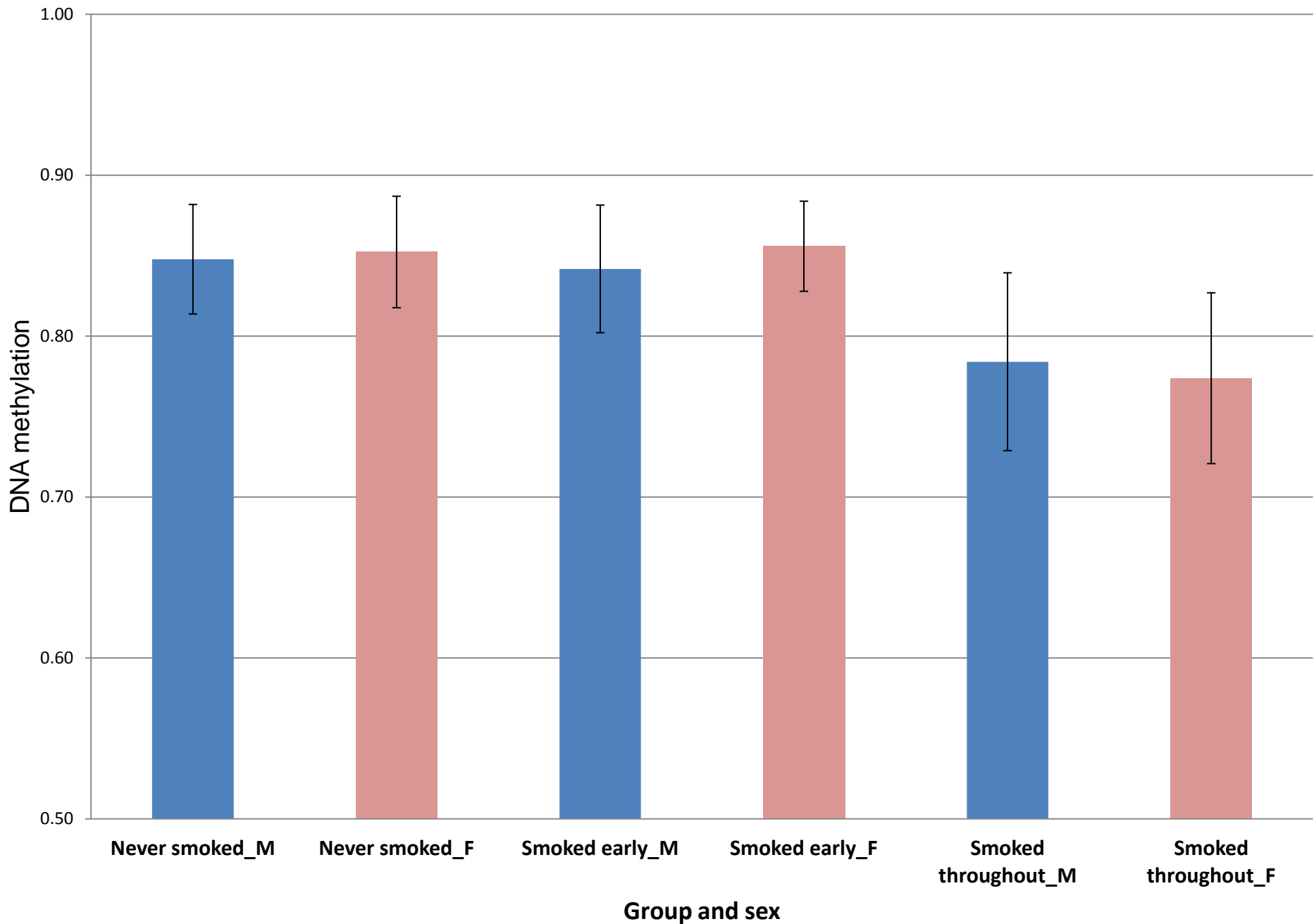
## Regional change in methylation – Buccal epithelium

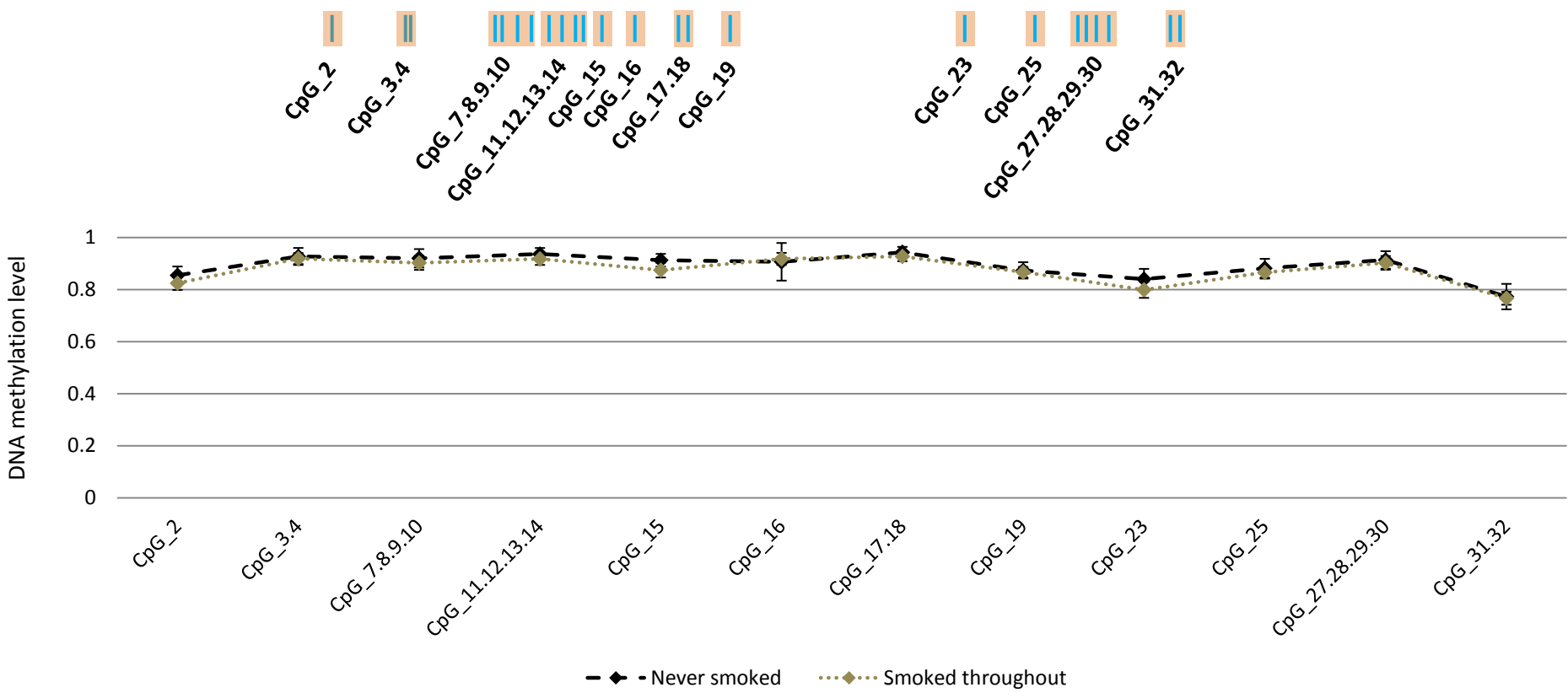
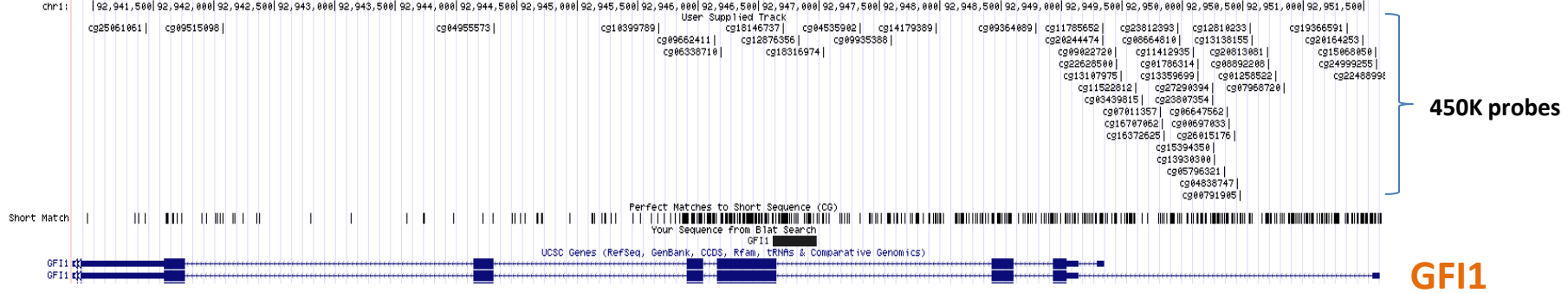


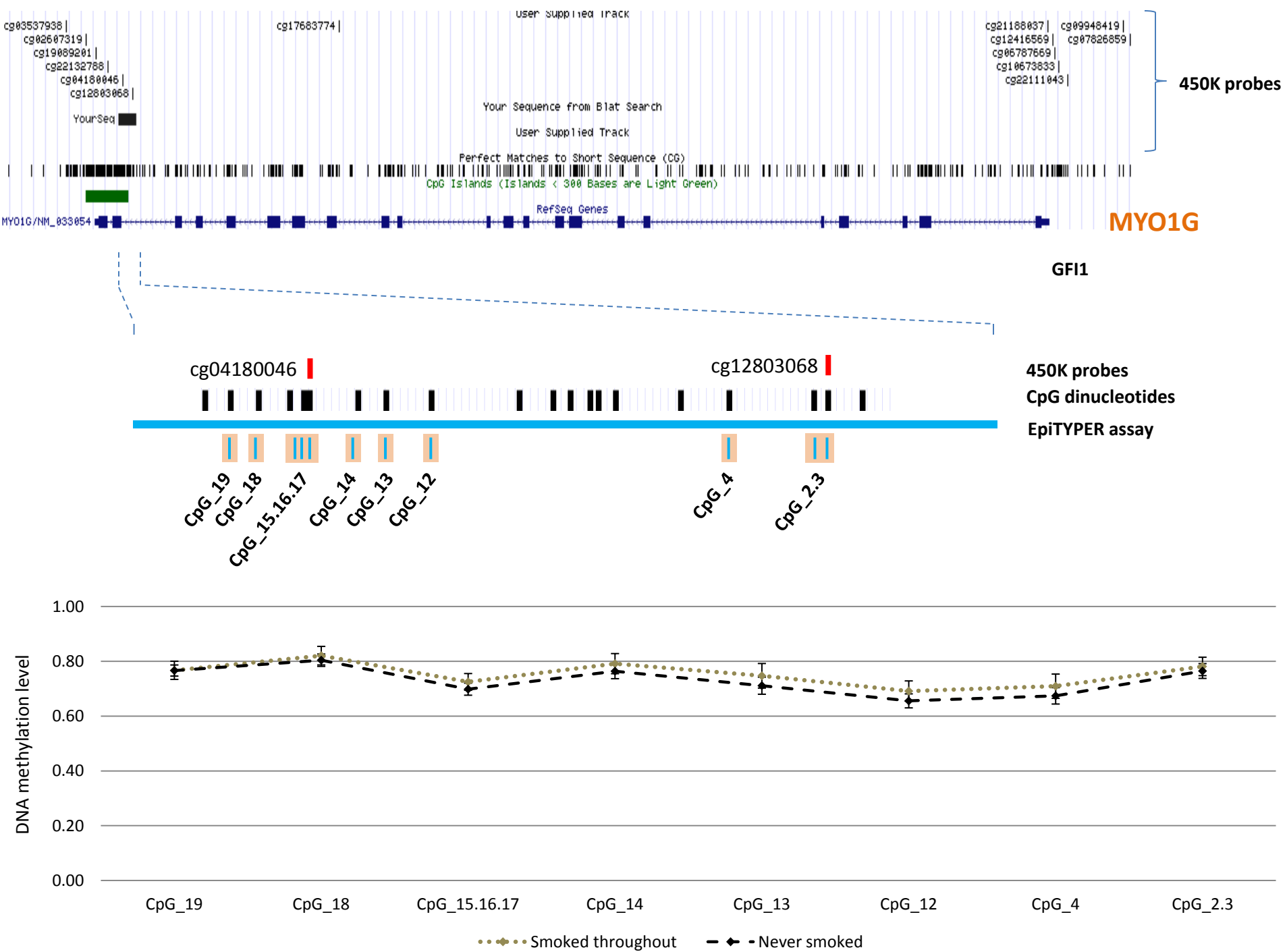
## Regional change in methylation - Placenta



# Assay A mean methylation in smokers and controls separated by sex



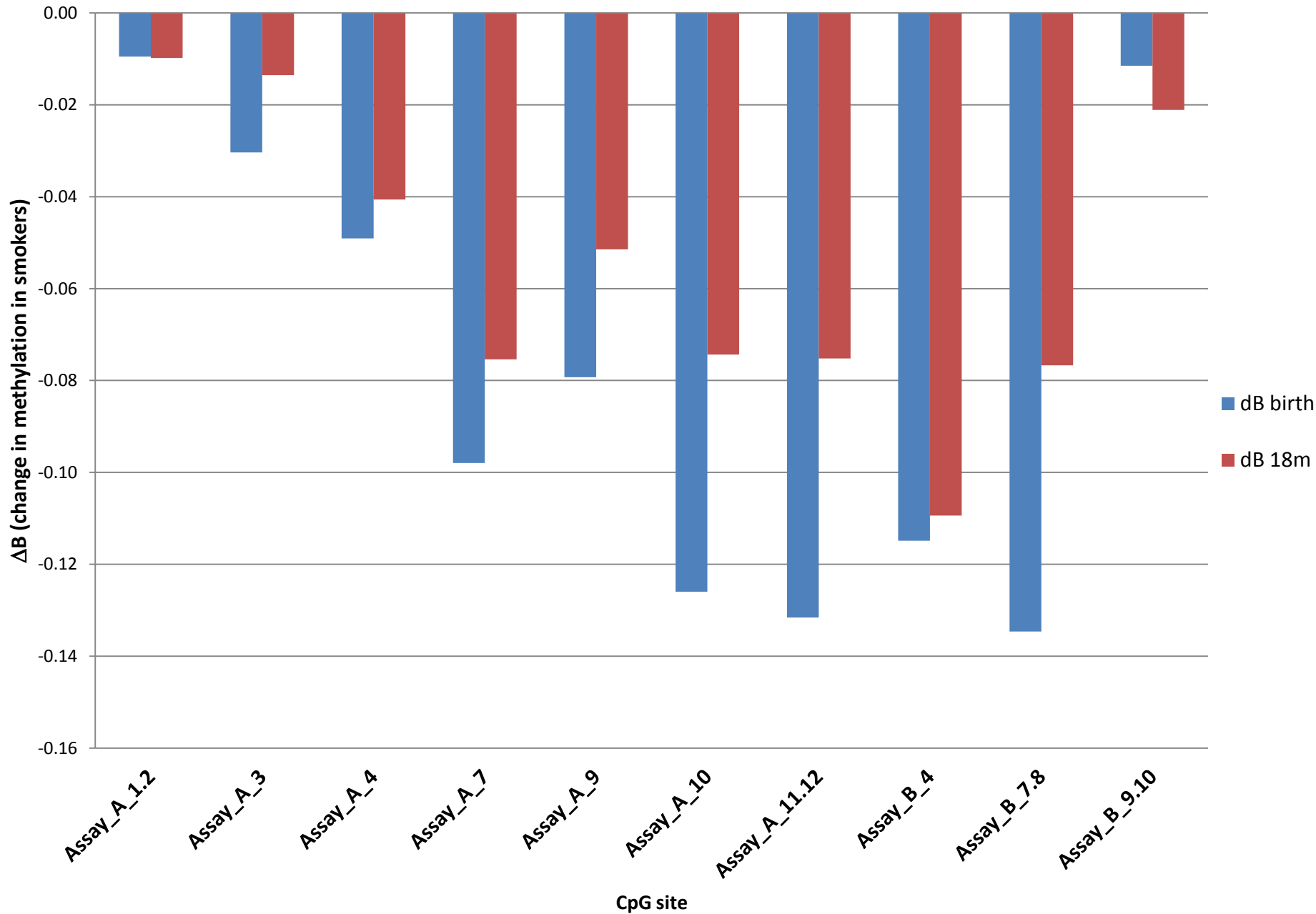








Difference in mean methylation between never smoked and smoked throughout groups at birth and 18m



## Supplementary file 1: EpiTYPER assay information

### Assay A

#### *Forward primer*

5' aggaagagagGTTGGTAATGGTTTTGAGATTTTT 3'

#### *Reverse primer*

5' cagtaatcagactcactataggagaaggctAAAACCAACCTATCCCCTACCTC 3'

#### *Target sequence*

5'GGCTGGTAATGGCCCTGAGACTCTCCAAGGTGGCTGAGGTTCTGCGCGGTTGGCAGAGTGCTGGCAGGAC  
ACAGGGGTTGCCAGGCTACAGATCCGGGTTCAGGCCAGCCGTGGGGACTGTTACCTCTGAGAGGGCAGC  
CCTGCCCCGCGCCCTGGCCCTGCCCTGCACCCGGCTGGGTCTCATCTGACACGCAGCCTTCCAGCCTCTCATT  
GCCCAGGGGTGGGCCCTGGGAGTGGTCTGGCAGGGCCCCTCTCTGCAGACCCTGCGGGACCAGCAGGCC  
GGGCGGTGGCTGGGAGGCAGGGGACAGGTTGGCCTC

### Assay B

#### *Forward primer*

5' aggaagagagAGTGGTTTTGGTAGGGTTTTTTTT 3'

#### *Reverse primer*

5' cagtaatcagactcactataggagaaggctAACCCCAATCTCCTCCTATAAT 3'

#### *Target sequence*

AGTGGTCTGGCAGGGCCCCTCTCTGCAGACCCTGCGGGACCAGCAGGCCGGGCGGTGGCTGGGAGGCAGG  
GGACAGGTTGGCCTCTGCGGTCTTGGAGGCACAGAGCTCCTCCGCCCCTGGGCCTGTGGCCCCCTCTGCTGG  
CCCAGCTGTGCCTTGTAAAGGGCCACGGTGATGGCCACCTTTCTACCAATGCCAGGAAGGACACCCGCTC  
CCAGCTCCCTTCTCCCATCCACTACCATGGCAACTGCGGTGGCTGGGCGGAGGGGGGTCTCTCCAC  
ATCACAGAGGAGGAGACTGGGGCT

### Assay C

#### *Forward primer*

5' aggaagagagTTTAGGTGGGATTTTTAGGTTTAGG 3'

#### *Reverse primer*

5' cagtaatcagactcactataggagaaggctATACAACCAACCCATTACAAAA 3'

#### *Target sequence*

CTCAGGTGGGACCCAGGCTCAGGGAGGGCGGGGGACCTGGGGAGGCCGTGGCCGGGTCCGCCGCC  
CGTGTGACCCGGAGCGCCGCGCGGACCCGAGCCCCATTCCCGCCACGCGCCTGGCGCTCCCCGCGCTCC  
TGGGGCTCTGGCGCCCTGCCCGGGCGGGCGGGGGCGGGAGCGCTGCTCGCACGCGAGGCCTGGCTGGCCC  
CATCGCGTGACGGCGCCGCGGCTGCGGGGAAGTAGGGGGGAAGTGGGCGGGCGCCCGGGGGGCAC  
GCGATGCCGGGAGGGGCGCGCCCTGCCGCCACCTACGCGTTCTCGGGGAAAAGGCCGGGGCTGCCGG  
GTCCGCGGGGCGCATTGCGGGTGGGACGGGGTGGCTCCGCTGCGGGAGCGACCCTGCAATGGGGCTTGG  
CTGCAC

**GFI1 assay**

***Forward primer***

5' aggaagagagTTAGTGATGGGTATATTGATTTTTT 3'

***Reverse primer***

5' cagtaatagactcactatagggagaaggctCCCTTCCTATAAAATCTCAAATA 3'

***Target sequence***

CCAGCGATGGGCACATTGACTTCTCCGAGGCTGTGGAGGCACAAGGGGAGCGTCCGGTCAGGCTTCAGACG  
GCAGAGCGGAGGCCGCCGGGCTGCGGCTGCGAGCGTGGCGTGTTCGCGGACTGCGGGGCACCCAGCGCTTG  
TAGAACACTGCGTGCCAGGTGCCAGGCTCGCCCCAGGGTAAAACGTGGCCCCGGCCCTGGGGCCTCGCA  
GGCCACTATAGGAGGCAGAACAGTAAACAGATCATTGAAAAGACCTCGGGAGAGGAGGTCGTAATTCTGT  
GCGAGTGCAGCGGAGGCGCTCGGCGTTCCGAGGATCTTTTCTGGCTGGACCCGCGCACCTGGAGATCTACA  
GGGAAGGG

**MYO1G assay**

***Forward primer***

5' aggaagagagGTTTTGTTGGATAGTAAGGATTTAGA 3'

***Reverse primer***

5' cagtaatagactcactatagggagaaggctTACAACACCACCAACTAATCTCCTC 3'

***Target sequence***

GGCCCTGTTGGACAGCAAGGATCTAGAACCATCAGTCCTGGAGAAAATCGTATTGTGCAGCGTCCCGCCAA  
GCTTCCCCTGTCAGCAGGGCCCCGAGAGGGAGGAGGGGGCGGTGGGGGGCTGGTGGCCCTGGCGAGGGC  
GGCGTCCCCGGGTGCGGTTGGGGTTCGGCAGAGGCTTCTGGGGAGGGGGTGAGTCCGGCTAGGCTGG  
GGTGCGGGTTGAGGCGGCTGCACTCTGACCCGCGCTCCGCTCAGGTGACGGGGCTGAGCGTGACCAGCGG  
AGGAGACCAGCTGGTGGTGTGCA

**Supplementary table 2.** Cotinine levels in maternal (28 week) and infant (birth/18 mth) plasma

Group	Maternal ID #	Maternal Cotinine conc (ug/L)	Infant birth ID #	Birth Cotinine conc (ug/L)	Infant 18 mths ID #	18 month Cotinine conc (ug/L)
Smoking	1-016	120	1016.1	85	1016.1	0.1
Smoking	2-028	126	2028.1	74	2028.1	0.5
Smoking	3-012	152	3012.2	11	3012.2	0
Non exposed	1-105	0	1105.2	0	1105.2	0
Non exposed	1-102	0	1102.2	0	1102.2	0
Non exposed	2-032	0	2032.2	0	2032.2	0

Smoked throughout	A_CpG_1.2	A_CpG_3	A_CpG_4	A_CpG_7	A_CpG_9	A_CpG_10	A_CpG_11.12	AVERAGE
1016.1C	0.95	0.81	0.90		0.67	0.70	0.63	0.78
1016.2C	0.93	0.80	0.78	0.67	0.66	0.64	0.62	0.73
1019.1C	0.95	0.82	0.87	0.66	0.68	0.65	0.62	0.75
1019.2C	0.96	0.87	0.81	0.67	0.64	0.64	0.65	0.75
1033.1C	0.97	0.80	0.94	0.86	0.79	0.85		0.86
1033.2C	0.97	0.83	0.92	0.89	0.83	0.89	0.88	0.88
1045.1C	0.96	0.86	0.92	0.79	0.77	0.79	0.81	0.84
1045.2C	0.93	0.85	0.85	0.73	0.70	0.65	0.67	0.77
1048.1C	0.96	0.89	0.94	0.80	0.76	0.82	0.77	0.85
1048.2C	0.96	0.88	0.92	0.86	0.81	0.80		0.87
1059.1C	0.96	0.86	0.90	0.76	0.77	0.80	0.74	0.82
1059.2C	0.96	0.85	0.79	0.62	0.63	0.54	0.53	0.70
2006.1C	0.95	0.86	0.85	0.75	0.72	0.68	0.67	0.78
2006.2C	0.93	0.86	0.85	0.71	0.60	0.63	0.60	0.74
2028.1C	0.93	0.84	0.84	0.70	0.71	0.65	0.67	0.76
2028.2C	0.96	0.84	0.85	0.75	0.68	0.74	0.65	0.78
2035.1C	0.95	0.84	0.80	0.67	0.63	0.61	0.60	0.73
2035.2C	0.96	0.83	0.79	0.69	0.60	0.63	0.61	0.73
2053.1C	0.96	0.87	0.90	0.82	0.78	0.78	0.77	0.84
2053.2C	0.95	0.85	0.89	0.76	0.76	0.68	0.70	0.80
3012.1C	0.92	0.79	0.79	0.71	0.57			0.75
3012.2C	0.93	0.78		0.69		0.71	0.70	0.76
3045.1C	0.93	0.85	0.84	0.64	0.60	0.60	0.61	0.72
3045.2C	0.94	0.80	0.79	0.72		0.63	0.57	0.74
Smoked throughout	0.95	0.84	0.86	0.73	0.70	0.70	0.67	0.78
ST DEV	0.01	0.03	0.05	0.07	0.08	0.09	0.08	0.05
95% CI	0.01	0.01	0.02	0.03	0.03	0.04	0.03	0.02

Smoked early	A_CpG_1.2	A_CpG_3	A_CpG_4	A_CpG_7	A_CpG_9	A_CpG_10	A_CpG_11.12	ASSAY_AVE
1018.1C	0.96	0.90	0.93		0.86	0.82	0.88	0.89
1018.2C	0.96	0.87	0.87		0.78	0.72	0.71	0.80
1055.1C	0.96	0.90	0.97		0.85	0.78	0.87	0.88
1055.2C	0.97	0.87	0.89		0.81	0.77	0.78	0.83

1072.1C									
1072.2C	0.95	0.88	0.90	0.92	0.83	0.91	0.89	0.90	
1074.1C	0.97	0.92	0.93	0.83	0.75	0.80	0.78	0.85	
1074.2C	0.95	0.89	0.94	0.87	0.77	0.87	0.85	0.88	
1140.1C									
1140.2C	0.96	0.87	0.94	0.87	0.76	0.78	0.78	0.85	
2039.1C	0.97	0.82	0.91	0.87	0.78	0.84	0.85	0.86	
2039.2C	0.97	0.82	0.93	0.87	0.80	0.84	0.85	0.87	
2054.1C	0.97	0.85	0.91	0.84	0.77	0.81	0.83	0.85	
2054.2C	0.95	0.80	0.90	0.85	0.81	0.84	0.83	0.85	
3007.1C	0.96	0.82	0.92	0.81	0.72	0.76	0.77	0.82	
3007.2C	0.96	0.85	0.92	0.76	0.75	0.73	0.77	0.82	
3008.1C	0.96	0.86	0.88	0.75	0.74	0.74	0.73	0.81	
3008.2C	0.96	0.86	0.86	0.72	0.67	0.71	0.70	0.78	
3018.1C	0.96	0.88	0.91	0.87	0.82	0.85	0.83	0.87	
3018.2C	0.97	0.87	0.93	0.91	0.84	0.90	0.90	0.90	
3034.1C	0.97	0.83	0.91	0.86	0.81	0.86	0.85	0.87	
3034.2C	0.95	0.77	0.88	0.84	0.80	0.85	0.86	0.85	
Smoked early	0.96	0.85	0.91	0.83	0.77	0.82	0.81	0.85	
SD	0.01	0.04	0.03	0.05	0.04	0.06	0.06	0.03	
95% CI	0.00	0.02	0.01	0.02	0.02	0.03	0.03	0.01	

Never smoked	A_CpG_1.2	A_CpG_3	A_CpG_4	A_CpG_7	A_CpG_9	A_CpG_10	A_CpG_11.12	AVERAGE	
1061.1C	0.95	0.85	0.91		0.74	0.73	0.74	0.70	0.80
1061.2C	0.95	0.91	0.72			0.72	0.79		0.82
1065.1C									
1065.2C	0.97	0.89	0.92		0.80	0.79	0.86	0.77	0.85
1069.1C	0.97	0.82	0.88		0.81	0.71	0.73	0.70	0.80
1069.2C	0.94	0.86	0.85		0.78	0.76	0.78	0.69	0.81
1082.1C	0.96	0.86	0.92		0.83	0.78	0.81	0.81	0.85
1082.2C	0.94	0.90	0.93		0.86	0.79	0.85	0.82	0.87
1099.1C	0.97	0.90	0.91		0.83	0.80	0.84	0.79	0.86
1099.2C	0.96	0.88	0.91		0.81	0.75	0.77	0.77	0.83
1102.1C	0.94		0.89		0.86	0.84	0.94	0.93	0.90
1102.2C	0.95	0.88	0.92		0.86	0.80	0.82	0.84	0.86

1106.1C	0.95	0.87	0.89	0.84	0.79	0.79	0.80	0.85
1106.2C	0.95	0.90	0.95	0.79	0.79	0.85	0.79	0.86
2019.1C	0.95	0.90	0.93	0.87	0.78	0.87	0.86	0.88
2019.2C	0.96	0.92	0.93	0.91	0.80	0.83	0.85	0.89
2038.1C	0.96	0.88	0.92	0.83	0.82	0.86	0.83	0.87
2038.2C	0.95	0.88	0.91	0.82	0.76	0.79	0.82	0.85
2065.1C	0.96	0.87	0.92	0.88	0.83	0.90	0.91	0.89
2065.2C	0.96	0.77	0.92			0.92	0.89	0.89
2073.1C	0.96	0.85	0.90	0.80	0.75	0.80	0.73	0.83
2073.2C	0.98	0.84	0.90	0.83	0.73	0.79	0.77	0.83
1053.1C	0.95	0.85	0.90	0.81	0.76	0.82	0.76	0.83
1053.2C	0.97		0.93	0.85	0.72	0.78	0.79	0.84
1062.1C	0.97	0.90	0.95	0.89	0.80	0.88	0.88	0.89
1062.2C	0.97	0.86	0.94	0.88	0.82	0.89	0.90	0.89
1068.1C	0.97	0.90	0.94	0.87	0.83	0.87	0.86	0.89
1068.2C	0.98	0.89	0.94	0.89	0.84	0.88	0.88	0.90
1092.1C	0.95	0.88	0.92	0.84	0.79	0.84	0.80	0.86
1092.2C	0.92	0.86	0.84	0.81	0.77	0.77	0.75	0.82
1093.1C	0.94	0.89	0.94	0.85	0.78	0.82	0.82	0.86
1093.2C	0.96	0.89	0.92	0.85	0.79	0.84	0.83	0.87
1105.1C	0.98	0.89	0.89	0.81	0.76	0.78	0.78	0.84
1105.2C	0.97	0.85	0.88	0.82	0.78	0.81	0.78	0.84
1110.1C	0.96	0.89	0.93	0.86	0.77	0.86	0.83	0.87
1110.2C	0.97	0.84	0.88	0.71	0.76	0.77	0.77	0.81
2032.1C	0.98	0.90	0.92	0.86	0.81	0.87	0.85	0.88
2032.2C	0.96	0.87	0.89	0.83	0.77	0.79	0.78	0.84
2045.1C	0.97	0.88	0.95	0.90	0.81	0.89	0.89	0.90
2045.2C	0.96	0.87	0.92	0.84	0.79	0.85	0.81	0.86
2056.1C	0.93	0.86		0.87	0.72	0.81	0.76	0.82
2056.2C	0.94	0.80	0.85		0.72		0.72	0.80
2064.1C	0.95	0.79	0.86	0.76	0.67		0.68	0.78
2064.2C	0.94	0.80		0.72		0.69	0.61	0.75
2071.1C	0.96	0.90	0.93	0.88	0.80	0.84	0.84	0.88
2071.2C	0.96	0.86	0.93	0.88	0.80		0.81	0.87
Never smoked	0.96	0.87	0.90	0.83	0.77	0.82	0.80	0.85



SD	0.01	0.03	0.04	0.05	0.04	0.05	0.07	0.03
95% CI	0.00	0.01	0.01	0.01	0.01	0.02	0.02	0.01