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### **Supplemental Material**

#### **Exposure to low levels of lead in utero and umbilical cord blood DNA methylation in Project Viva: an epigenome-wide association study**

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**Table S1. Epigenetics blood sample size flow in Project Viva**

	Cord blood	Mid-childhood
<b>Initial</b>	507	473
QC (quality control)		
Low quality	10	6
Sex mismatch	6	3
Genotype mismatch	6	4
Total removed QC	22	13
<b>Total after QC</b>	485	460
No maternal lead exposure	216	219
Undetectable maternal blood lead	1	1
<b>Available for statistical analysis</b>	268	240

**Table S2. Maternal red blood cell lead levels by infant sex ( $\mu\text{g/dL}$ ).**

	N	Mean	SD	Min	Max	Percentiles						
						1 <sup>st</sup>	5 <sup>th</sup>	25 <sup>th</sup>	50 <sup>th</sup>	75 <sup>th</sup>	95 <sup>th</sup>	99 <sup>th</sup>
Male	130	1.19	0.63	0.36	4.97	0.42	0.57	0.80	1.04	1.41	2.58	3.03
Female	138	1.24	0.63	0.29	4.93	0.39	0.55	0.84	1.11	1.44	2.33	3.55

**Table S3. CpGs with DNA methylation levels in blood samples in mid-childhood associated with prenatal maternal red blood cell lead concentrations in analyses for all children, males and females (loci identified from EWAS for cord blood).**

CpG	Chr	Position	Relation to island	Gene	Relation to gene	Average methylation (%) <sup>a</sup>	All children		Males		Females		<i>p</i> value for interaction
							Effect estimate (SE) <sup>b</sup>	<i>p</i> value	Effect estimate (SE) <sup>b</sup>	<i>p</i> value	Effect estimate (SE) <sup>b</sup>	<i>p</i> value	
<b>Top loci identified for all newborns</b>													
cg02272457	chr6	42427790	Open Sea	--	--	99.2	-0.01 (0.02)	5.4E-01	-0.04 (0.02)	2.9E-02	0.04 (0.03)	2.7E-01	0.01
cg20324491	chr7	149128397	N_Shore	--	--	94.2	-0.03 (0.1)	7.5E-01	0.0 (0.2)	9.6E-01	-0.1 (0.2)	6.2E-01	0.44
cg22112000	chr7	5647182	Island	--	--	98.7	-0.01 (0.01)	4.0E-01	-0.03 (0.0)	2.0E-01	0.01 (0.02)	6.3E-01	0.62
cg10773601	chr19	51226046	N_Shore	<i>CLEC11A</i>	TSS1500	86.4	-0.5 (0.3)	1.5E-01	-0.21 (0.41)	6.1E-01	-0.7 (0.5)	1.6E-01	0.14
<b>Top loci identified for male infants</b>													
cg22512536	chr5	154134507	Island	<i>LARPI</i>	Body	1.9	-0.03 (0.04)	4.6E-01	-0.1 (0.04)	4.8E-02	0.03 (0.05)	5.2E-01	0.16
cg08964024	chr12	14923326	Island	--	--	1.6	-0.001 (0.03)	9.6E-01	-0.01 (0.03)	8.7E-01	0.01 (0.04)	7.4E-01	0.99
<b>Top loci identified for female infants</b>													
cg04295372	chr1	101184332	Open Sea	<i>VCAM1</i>	TSS1500	51.8	-0.02 (0.3)	9.6E-01	0.1 (0.5)	9.1E-01	-0.3 (0.4)	4.3E-01	0.97
cg09356083	chr1	2430057	N_Shelf	<i>PLCH2</i>	Body	86.3	-0.1 (0.1)	2.3E-01	-0.3 (0.2)	1.1E-01	-0.004 (0.1)	9.8E-01	0.60
cg20816789	chr1	27462916	Open Sea	<i>SLC9A1</i>	Body	86.3	0.1 (0.1)	5.8E-01	0.1 (0.2)	4.6E-01	0.1 (0.2)	4.8E-01	0.94
cg22446399	chr2	27326580	Open Sea	<i>CGREF1</i>	Body	96.4	0.04 (0.1)	7.2E-01	-0.01 (0.2)	9.6E-01	0.1 (0.1)	4.8E-01	0.97
cg27403609	chr2	11101403	Island	--	--	92.7	-0.1 (0.3)	7.3E-01	0.1 (0.4)	9.0E-01	-0.4 (0.4)	3.9E-01	0.17
cg07248017	chr4	8230689	Open Sea	<i>SH3TC1</i>	Body	62.4	0.1 (0.2)	7.2E-01	0.2 (0.3)	5.2E-01	-0.01 (0.3)	9.7E-01	0.40
cg06239355	chr5	32714010	Island	<i>NPR3</i>	Body	2.1	0.01 (0.03)	8.3E-01	0.03 (0.05)	5.5E-01	-0.02 (0.05)	6.3E-01	0.54
cg15601915	chr5	1065775	S_Shore	<i>SLC12A7</i>	Body	84.6	-0.01 (0.2)	9.4E-01	0.4 (0.2)	1.1E-01	-0.3 (0.2)	1.5E-01	0.01
cg17599748	chr6	31589597	S_Shore	<i>SNORA38</i>	TSS1500	72.8	0.1 (0.3)	8.2E-01	-0.04 (0.3)	9.1E-01	0.1 (0.5)	9.1E-01	0.60
cg08673909	chr7	1329592	Island	--	--	3.3	0.03 (0.1)	7.3E-01	0.2 (0.1)	6.5E-02	-0.2 (0.1)	1.4E-01	0.01
cg10090217	chr7	45151583	S_Shore	<i>TBRG4</i>	TSS1500	2.1	0.04 (0.02)	4.3E-02	0.1 (0.02)	6.6E-03	-0.001 (0.03)	9.8E-01	0.11
cg16565528	chr8	91010920	N_Shelf	--	--	79.3	0.06 (0.4)	8.8E-01	-0.04 (0.5)	9.3E-01	0.2 (0.5)	6.5E-01	0.81
cg03152353	chr9	139417194	Island	<i>NOTCH1</i>	Body	64.2	0.3 (0.2)	2.3E-01	0.4 (0.4)	3.0E-01	-0.04 (0.3)	8.8E-01	0.46
cg13817920	chr9	96587843	N_Shore	--	--	92.0	0.2 (0.1)	1.3E-01	0.2 (0.2)	2.4E-01	0.2 (0.2)	3.9E-01	0.86
cg13791644	chr10	119794366	Open Sea	<i>RAB11FIP2</i>	Body	94.2	0.1 (0.2)	6.0E-01	0.2 (0.2)	2.4E-01	-0.04 (0.3)	9.1E-01	0.46
cg18454045	chr10	88391658	N_Shore	--	--	72.9	0.2 (0.3)	4.3E-01	0.6 (0.4)	2.0E-01	-0.1 (0.4)	8.7E-01	0.32
cg11127561	chr11	125462151	N_Shore	<i>STT3A</i>	TSS1500	1.5	-0.02 (0.02)	3.9E-01	0.004 (0.03)	9.0E-01	-0.04 (0.03)	1.9E-01	0.46
cg17971003	chr11	107582804	OpenSea	<i>SLN</i>	TSS200	93.9	0.05 (0.2)	7.9E-01	-0.1 (0.2)	6.6E-01	0.3 (0.3)	3.0E-01	0.61
cg24637308	chr11	6592297	Island	<i>DNHD1</i>	Body	79.9	-2.5 (0.9)	4.7E-03	-1.3 (1.5)	3.5E-01	-4.1 (1.4)	2.7E-03	0.13
cg05959994	chr13	114253916	Open Sea	<i>TFDP1</i>	Body	98.5	-0.03 (0.04)	4.6E-01	-0.1 (0.1)	3.8E-01	-0.05 (0.1)	4.8E-01	0.93
cg11203293	chr13	25777762	Open Sea	--	--	68.1	-0.7 (0.6)	2.4E-01	-0.2 (0.9)	7.8E-01	-1.3 (0.8)	9.8E-02	0.59
cg04545835	chr14	102675570	Open Sea	<i>WDR20</i>	Body	97.2	0.01 (0.1)	9.2E-01	-0.2 (0.1)	8.8E-02	0.2 (0.1)	3.6E-02	0.06
cg08131309	chr15	40399131	N_Shore	<i>BMF</i>	TSS1500	34.4	0.1 (0.2)	7.2E-01	0.3 (0.3)	3.2E-01	-0.2 (0.3)	5.3E-01	0.56
cg04730825	chr16	16116191	Open Sea	<i>ABCC1</i>	Body	33.0	-0.1 (0.1)	3.3E-01	0.1 (0.2)	6.4E-01	-0.4 (0.2)	6.2E-02	0.24
cg26686608	chr16	88705716	N_Shore	<i>IL17C</i>	Body	42.3	0.5 (0.3)	9.8E-02	0.3 (0.5)	5.9E-01	0.6 (0.4)	1.6E-01	0.73
cg00461015	chr17	42295635	N_Shore	<i>UBTF</i>	5'UTR	1.8	-0.04 (0.03)	1.5E-01	-0.004 (0.03)	9.1E-01	-0.1 (0.04)	8.9E-02	0.21
cg04571282	chr17	44108753	Open Sea	<i>KIAA1267</i>	3'UTR	98.3	-0.1 (0.03)	3.0E-02	-0.1 (0.04)	2.5E-02	-0.04 (0.1)	4.3E-01	0.58
cg11252953	chr17	80358829	N_Shelf	<i>C17orf101</i>	Body	6.2	-0.1 (0.2)	4.3E-01	-0.3 (0.2)	2.5E-01	0.1 (0.3)	6.0E-01	0.61
cg15922057	chr17	25784714	S_Shore	--	--	27.4	-0.1 (0.3)	8.3E-01	-0.3 (0.4)	4.9E-01	0.2 (0.5)	5.9E-01	0.55
cg06753949	chr19	15334309	Island	--	--	1.9	-0.03 (0.04)	5.0E-01	-0.04 (0.1)	5.2E-01	-0.04 (0.1)	5.1E-01	0.60
cg07780528	chr19	35630334	N_Shelf	<i>FXYD1</i>	5'UTR	32.9	-0.2 (0.2)	4.7E-01	0.3 (0.4)	3.7E-01	-0.5 (0.3)	7.6E-02	0.13
cg11610754	chr19	18722595	Island	<i>TMEM59L</i>	TSS1500	1.8	0.04 (0.02)	1.2E-01	0.1 (0.03)	8.8E-02	0.02 (0.03)	6.1E-01	0.53
cg18598117	chr19	941126	Island	<i>ARID3A</i>	Body	23.4	-0.6 (0.5)	2.5E-01	-0.4 (0.7)	5.1E-01	-0.7 (0.8)	3.8E-01	0.83

cg22217660	chr19	50765301	S_Shelf	<i>MYH14</i>	Body	94.3	-0.3 (0.2)	1.6E-01	-0.3 (0.3)	3.3E-01	-0.3 (0.2)	1.4E-01	0.62
cg03373781	chr20	30582113	N_Shore	<i>XKR7</i>	Body	70.7	-0.1 (0.2)	5.7E-01	-0.1 (0.3)	8.3E-01	-0.1 (0.3)	8.0E-01	0.88
cg07341934	chr20	57463711	Island	<i>GNAS</i>	3'UTR	57.5	-0.1 (0.4)	7.8E-01	0.2 (0.5)	7.3E-01	-0.3 (0.5)	5.0E-01	0.32
cg21307155	chr20	3216740	N_Shore	<i>SLC4A11</i>	Body	83.7	-0.1 (0.2)	6.5E-01	0.03 (0.2)	9.0E-01	-0.04 (0.3)	8.8E-01	0.94
cg17174023	chr22	50987453	Island	<i>KLHDC7B</i>	1stExon	9.5	-0.01 (0.3)	9.7E-01	0.7 (0.4)	1.1E-01	-0.7 (0.4)	8.7E-02	0.11

Abbreviations: Chr, Chromosome; FDR, false discovery rate; SE, standard error; TSS200, within 200 bps from transcription start site; TSS1500, within 1500 bps from transcription start site; Body, gene body; UTR, untranslated regions.

<sup>a</sup> Average methylation in blood samples in mid-childhood.

<sup>b</sup> The percent change in DNA methylation level for every doubling increase in prenatal maternal red blood cell lead concentrations. Adjusted for child sex, maternal age, maternal pre-pregnancy BMI, maternal race, maternal educational level, maternal smoking status, parity, gestational age, child age at sample collection and differential nucleated cell proportions (monocytes, CD8+ T cells, CD4+ T cells, Natural Killer cells and B cells).

**Table S4 to Table S12 were submitted as EXCEL tables separately.**

**Table S13. The associations between prenatal maternal red blood cell lead concentrations and umbilical cord blood CpGs identified by Engström et al. (2015) in analyses for all newborns in the present study.**

CpG	Chr	Position	Relation island to	Gene	Relation gene to	Average methylation (%) <sup>a</sup>	Effect estimate (SE) <sup>b</sup>	<i>p</i> value
cg18355337	19	55549722	Open Sea	<i>GP6</i>	TSS200	65.0	1.7 (1.2)	1.6E-01
cg25196158	1	214152979	N_Shore	--	--	5.0	-0.1 (0.1)	5.8E-01
cg16943697	2	120280763	N_Shore	<i>SCTR</i>	Body	51.3	0.1 (0.2)	7.2E-01
cg03833077	19	10024709	Island	<i>OLFM2</i>	Body	31.4	0.1 (0.3)	7.2E-01
cg12504721	5	138897583	Island	--	--	2.1	0.04 (0.03)	2.3E-01
cg23796967	19	55549590	Open Sea	<i>GP6</i>	1st Exon	7.6	0.1 (0.6)	9.0E-01
cg00145875	2	11104394	S_Shelf	--	--	83.2	0.4 (0.2)	1.0E-01
cg25818583	19	55549801	Open Sea	<i>GP6</i>	TSS200	74.1	-0.1 (0.6)	8.8E-01
cg23173307	4	786244	Island	<i>CPLX1</i>	Body	2.0	-0.05 (0.03)	7.6E-02
cg04942107	10	3918567	Open Sea	--	--	92.8	-0.1 (0.1)	5.4E-01
cg05374025	19	55549746	Open Sea	<i>GP6</i>	TSS200	50.0	2.0 (0.9)	3.1E-02
cg26668675	6	31148463	Island	--	--	55.6	0.1 (0.7)	9.0E-01
cg25472897	8	145560555	Island	<i>SCRT1</i>	TSS1500	20.5	-0.1 (0.3)	7.1E-01
cg00436174	2	128051630	Island	<i>ERCC3</i>	1st Exon	7.8	0.2 (0.1)	6.3E-02
cg11790196	14	89995679	Open Sea	<i>FOXN3</i>	5'UTR	96.9	0.01 (0.1)	9.2E-01
cg01519017	3	61227050	Open Sea	<i>FHIT</i>	5'UTR	97.7	-0.1 (0.05)	6.5E-02
cg16653408	22	42313569	N_Shore	--	--	79.9	-0.2 (0.2)	2.2E-01
cg03978169	6	33091357	Open Sea	<i>HLA-DPB2</i>	Body	95.9	-0.02 (0.1)	8.8E-01
cg18461584	7	55304314	Open Sea	--	--	95.8	0.1 (0.1)	2.0E-01
cg23352003	18	24237245	Island	--	--	7.1	0.01 (0.2)	9.4E-01
cg07313720	2	209029594	Open Sea	<i>CRYGA</i>	TSS1500	95.1	-0.1 (0.1)	6.7E-01
cg04432660	7	4802132	Island	<i>FO XK1</i>	3'UTR	98.6	-0.01 (0.02)	4.5E-01
cg04606773	10	134759693	S_Shelf	--	--	97.4	0.02 (0.1)	8.0E-01
cg22994018	11	105448797	Open Sea	--	--	78.3	-0.5 (0.8)	5.3E-01
cg25979157	19	53902687	S_Shelf	<i>ZNF765</i>	Body	98.5	-0.02 (0.04)	5.8E-01
cg11774346	21	46797899	N_Shelf	--	--	96.3	-0.02 (0.1)	8.0E-01
cg11668188	1	7891116	S_Shelf	<i>PER3</i>	Body	94.7	0.1 (0.2)	7.2E-01
cg25192902	11	121163419	Island	<i>SC5DL</i>	5'UTR	1.4	0.02 (0.02)	1.3E-01
cg24737570	2	11727173	Open Sea	<i>GREB1</i>	Body	86.5	-0.003 (0.2)	9.8E-01
cg05749559	8	36793457	Open Sea	<i>KCNU1</i>	3'UTR	92.4	0.1 (0.2)	7.6E-01



Abbreviations: Chr, Chromosome; FDR, false discovery rate; SE, standard error; TSS200, within 200 bps from transcription start site; TSS1500, within 1500 bps from transcription start site; Body, gene body; UTR, untranslated regions.

<sup>a</sup> Average methylation in umbilical cord blood among all newborns.

<sup>b</sup> The percent change in DNA methylation level for every doubling increase in prenatal maternal red blood cell lead concentrations. Adjusted for child sex, maternal age, maternal pre-pregnancy BMI, maternal race, maternal educational level, maternal smoking status, parity, gestational age and differential nucleated cell proportions (monocytes, CD8+ T cells, CD4+ T cells, Natural Killer cells, B cells and nucleated red blood cells).

**Table S14. Differentially methylated regions in cord blood associated with prenatal maternal red blood cell lead concentrations in analyses for all children, males and females (selected based on FWER<0.25 in bump hunting analysis).**

Chr	Start <sup>a</sup>	End <sup>a</sup>	Gene	Effect estimate <sup>b</sup>	Area <sup>c</sup>	No. of CpGs	<i>p</i> value	FWER
<b>All newborns</b>								
chr13	50194554	50194643	--	-9.20	0.185	2	6.50E-06	0.027
chr5	1594282	1594863	SDHAP3	-4.00	0.404	10	4.01E-05	0.159
chr12	31271429	31272119	--	-5.20	0.261	5	5.38E-05	0.196
chr6	32632159	32632338	HLA-DQB1	5.90	0.235	4	5.29E-05	0.202
chr19	12876846	12877188	HOOK2	5.70	0.230	4	6.50E-05	0.244
<b>Male infants</b>								
chr6	29648271	29649084	--	-4.70	1.040	22	6.50E-06	0.028
chr5	135415819	135416613	--	6.00	0.783	13	1.09E-05	0.047
chr6	32551749	32552205	HLA-DRB1	-5.80	0.634	11	4.32E-05	0.165
<b>Female infants</b>								
chr19	13875014	13875137	--	8.90	0.266	3	7.27E-05	0.228

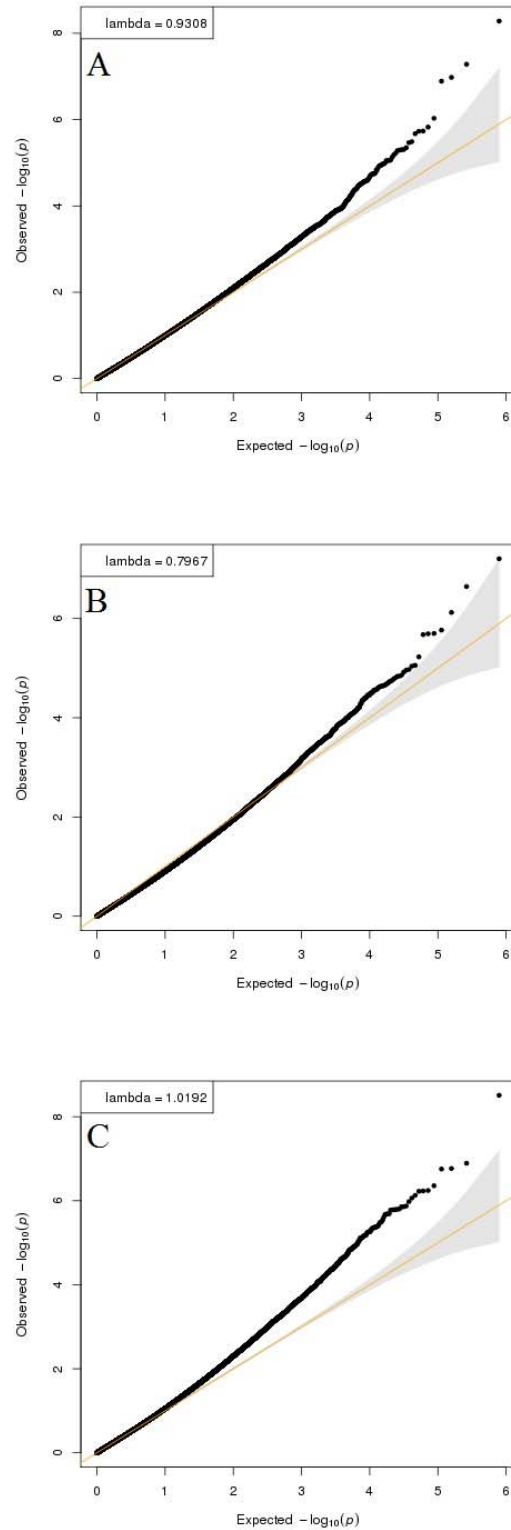
Abbreviations: Chr, Chromosome; FWER, family-wise error rate.

<sup>a</sup> Position of start and end of the differentially methylated region.

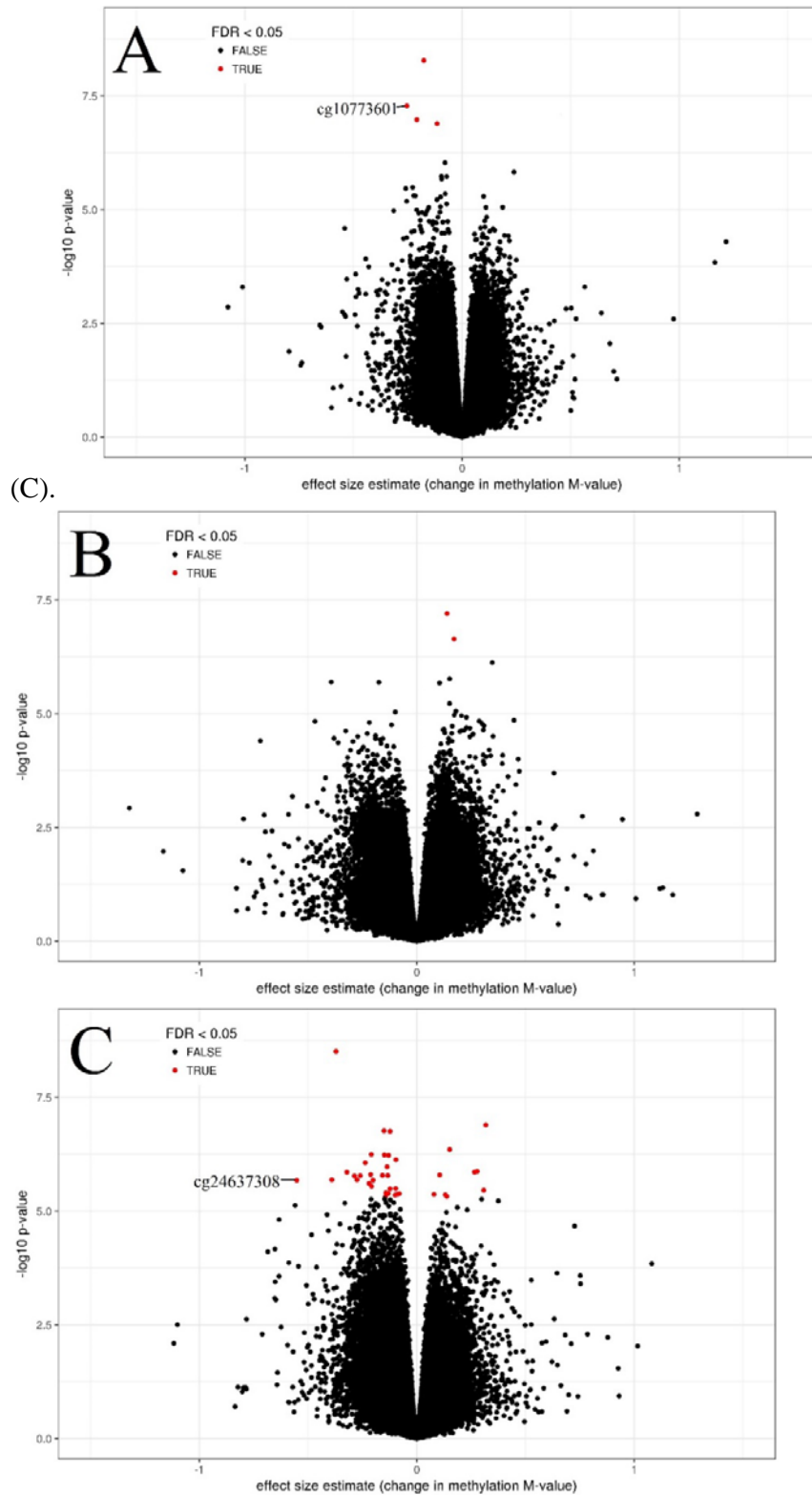
<sup>b</sup> The percent change in DNA methylation level for every doubling increase in prenatal maternal red blood cell lead concentrations. Adjusted for child sex, maternal age, maternal pre-pregnancy BMI, maternal race, maternal educational level, maternal smoking status, parity, gestational age and differential nucleated cell proportions (monocytes, CD8+ T cells, CD4+ T cells, Natural Killer cells, B cells and nucleated red blood cells).

<sup>c</sup> The strength of evidence for each differentially methylated region is summarized with its area.

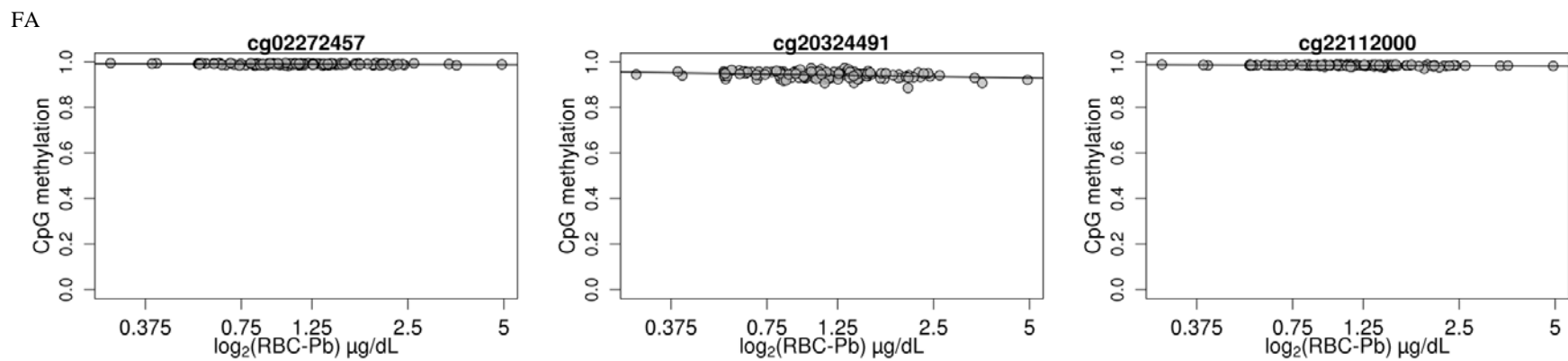
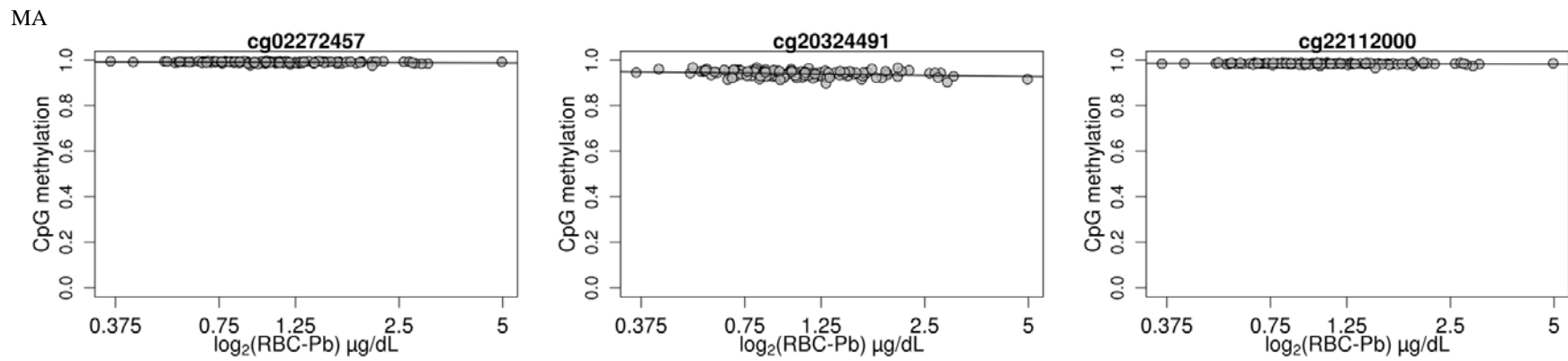
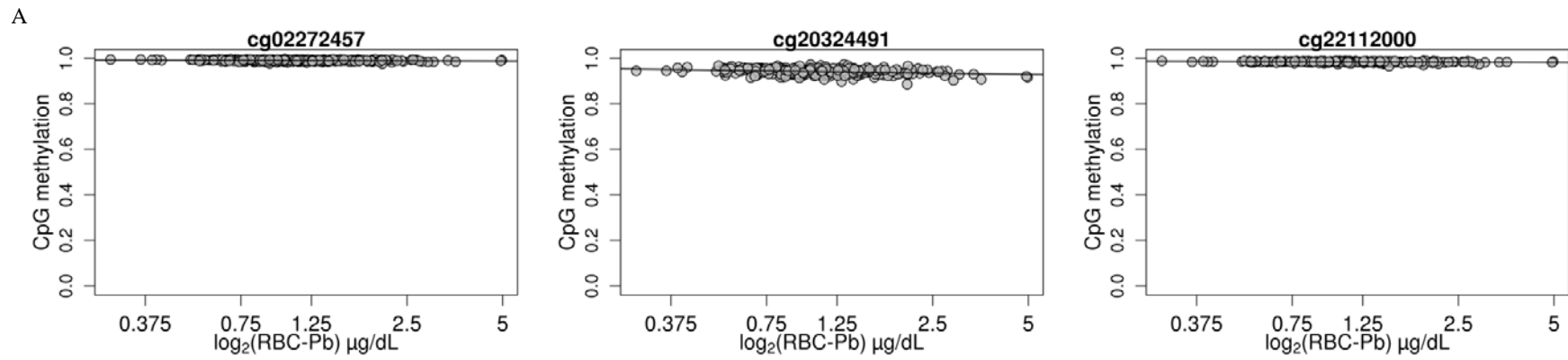
**Figure S1.** Quantile-quantile plots of the associations between prenatal maternal red blood cell lead concentrations and DNA methylation at 394,460 loci in umbilical cord blood among all newborns (A), male infants (B) and female infants (C).



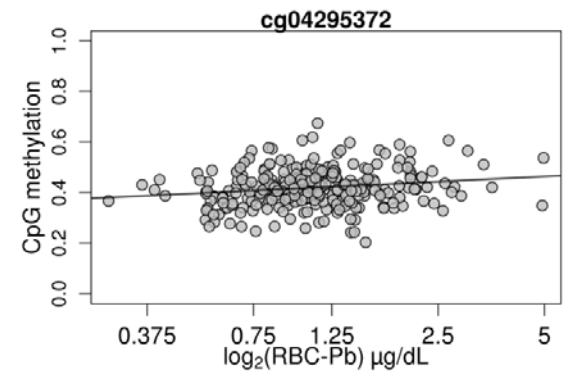
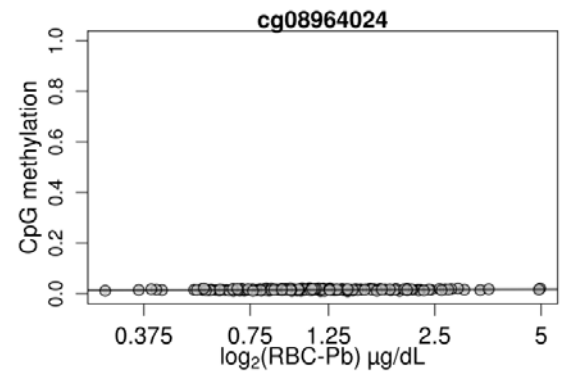
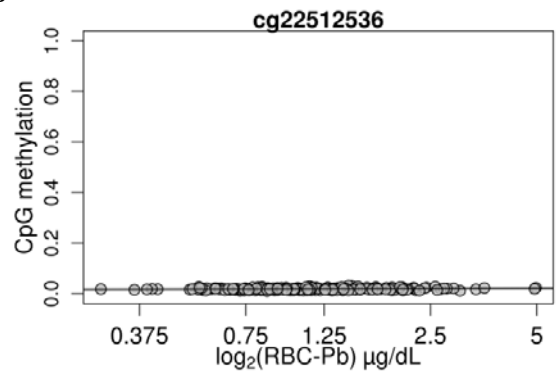
**Figure S2.** Volcano plots of the associations between prenatal maternal red blood cell lead concentrations and DNA methylation at 394,460 loci in cord blood among all newborns (A), male infants (B) and female infants



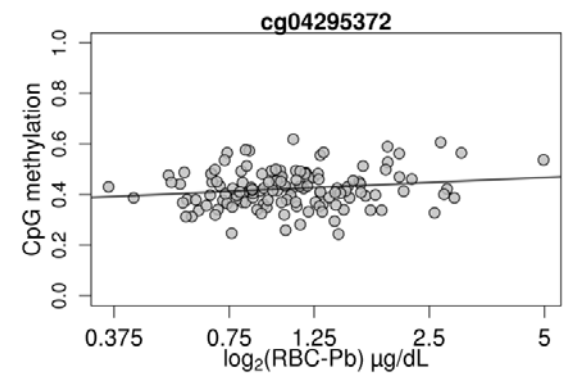
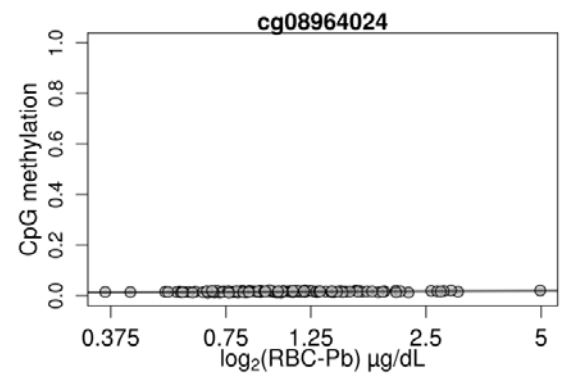
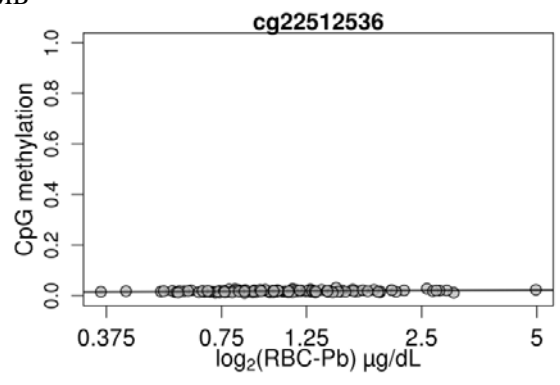
**Figure S3.** Scatterplots for prenatal red blood cell lead levels and methylation levels at top loci in umbilical cord blood DNA among all newborns (A-N, upper panel in each page), male infants (MA-MN, middle panel in each page) and female infants (FA-FN, lower panel in each page).



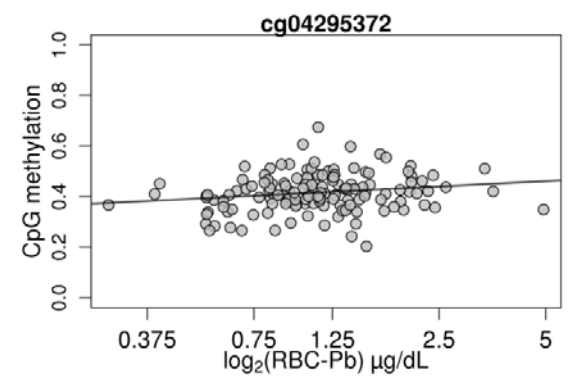
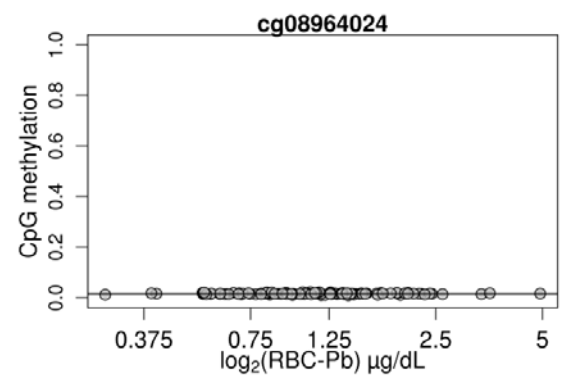
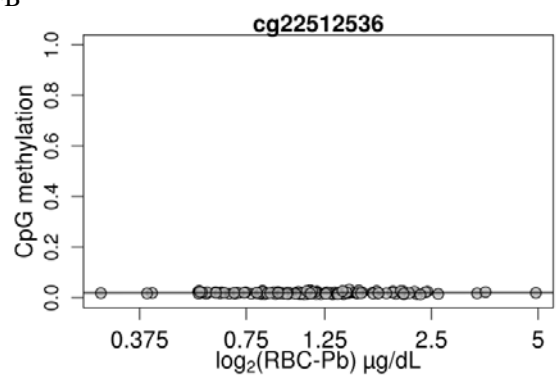
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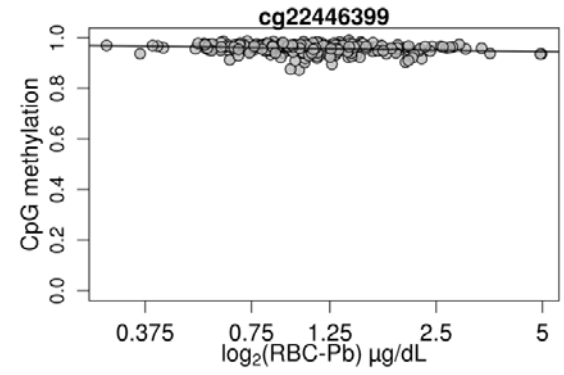
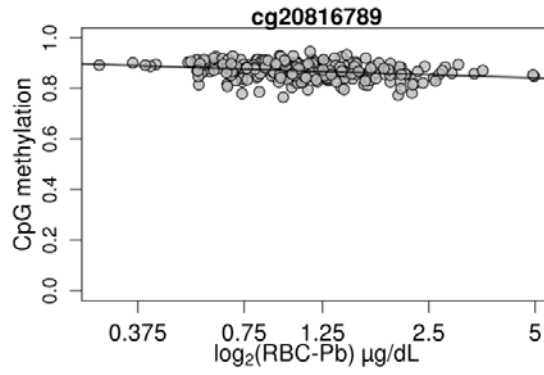
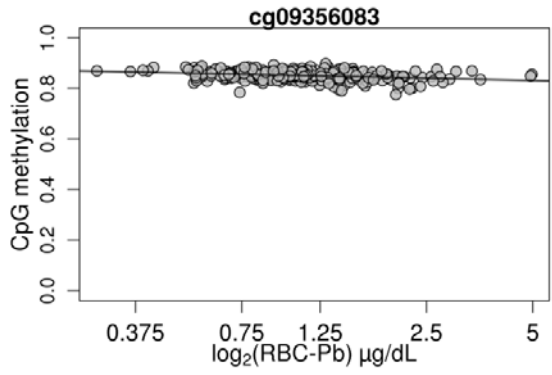
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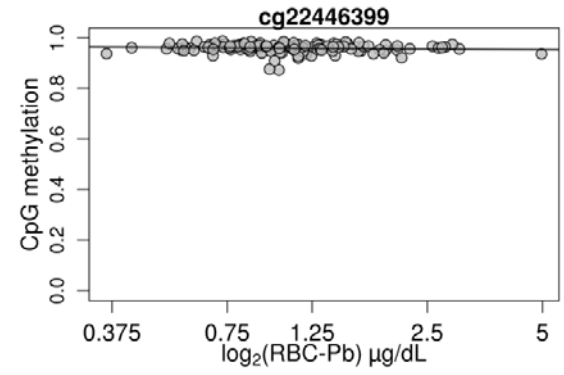
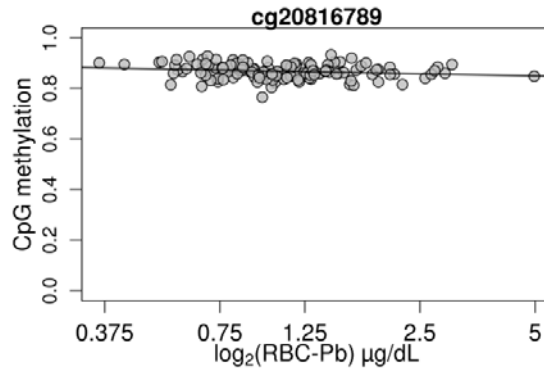
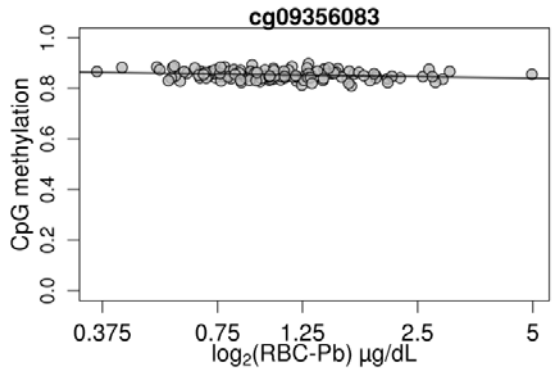
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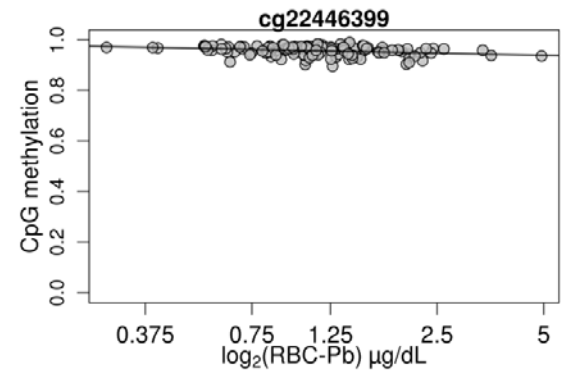
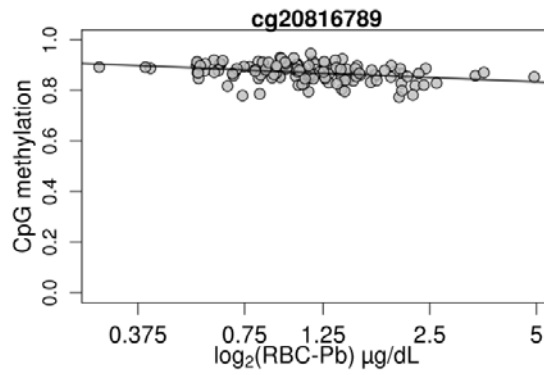
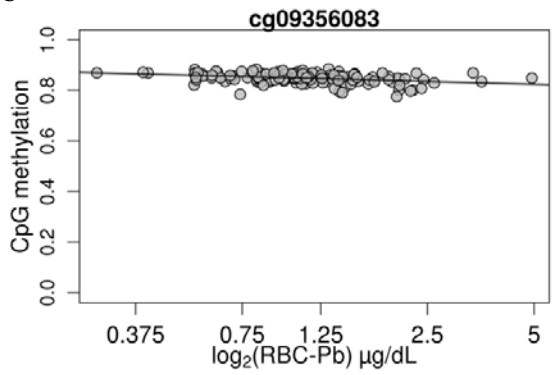
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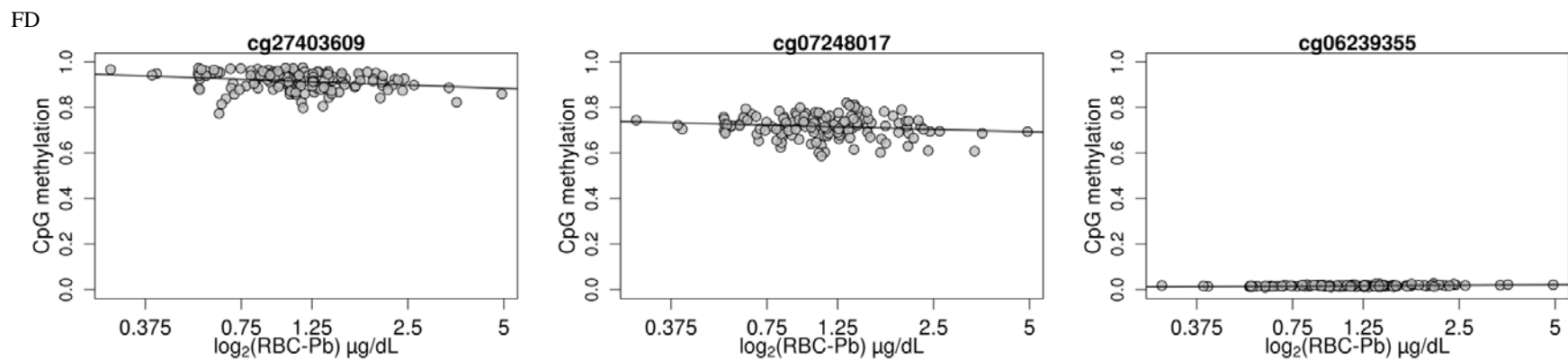
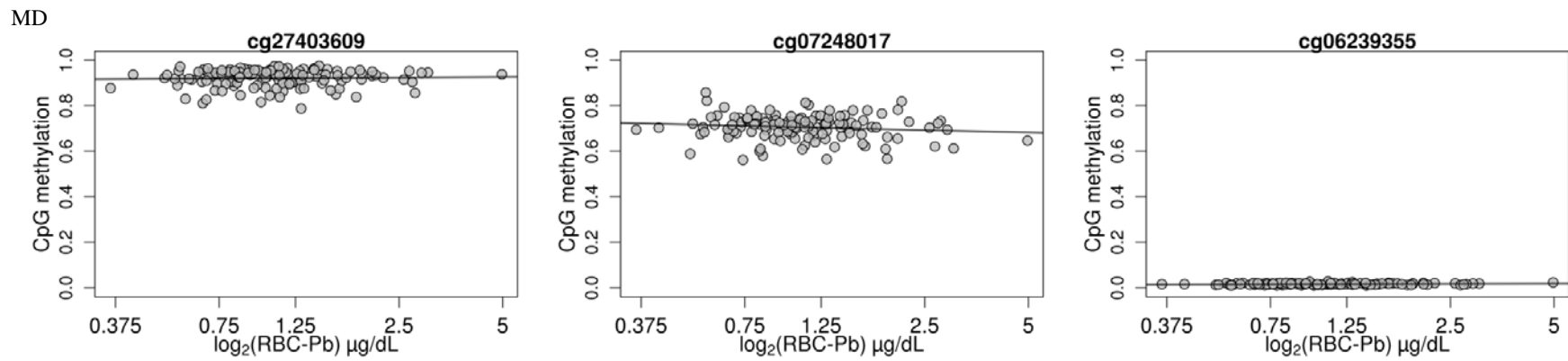
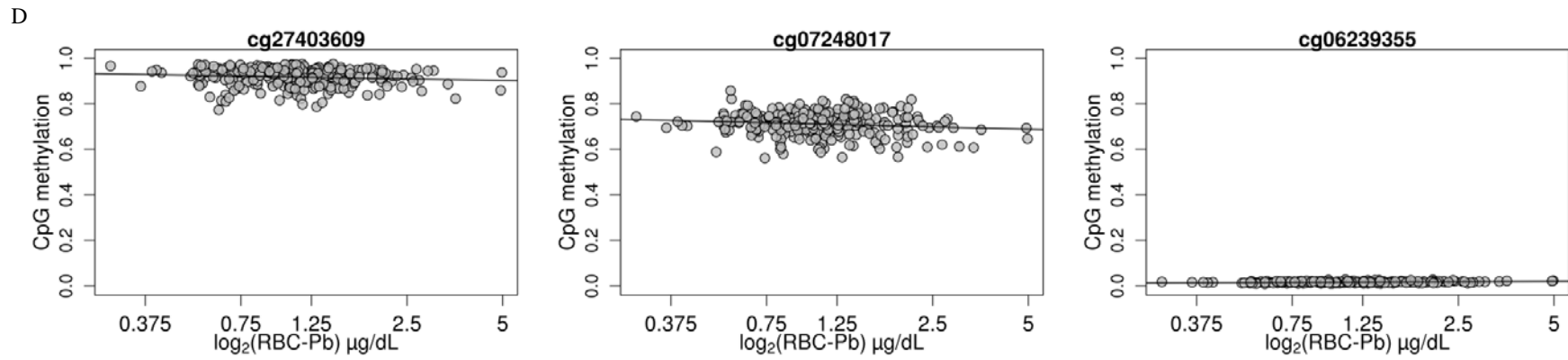
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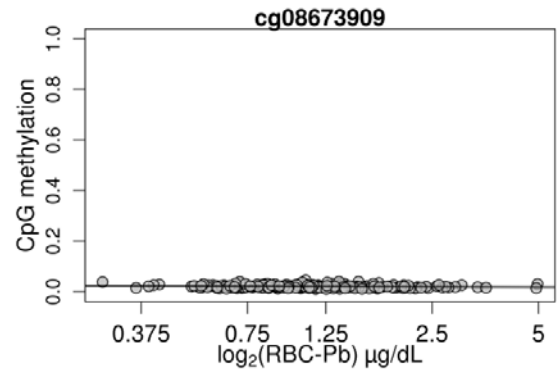
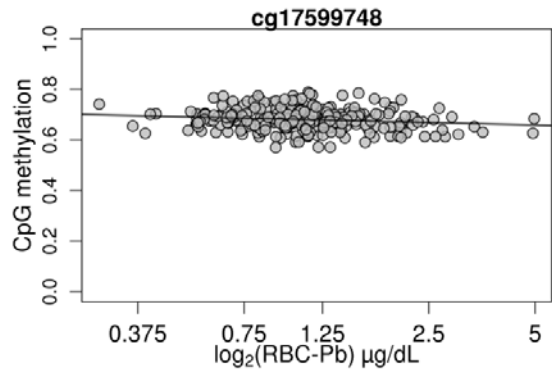
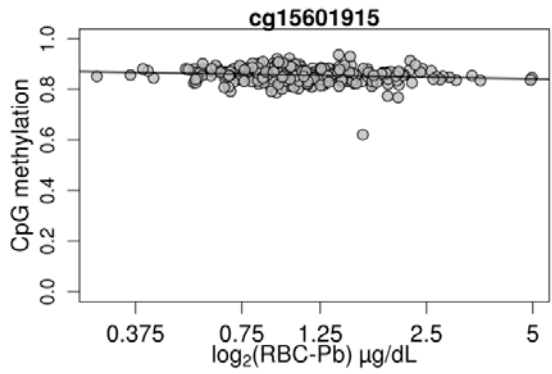
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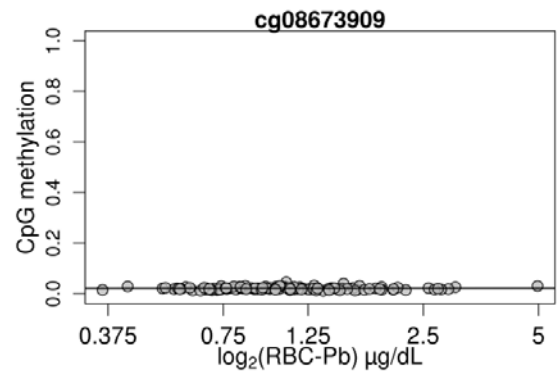
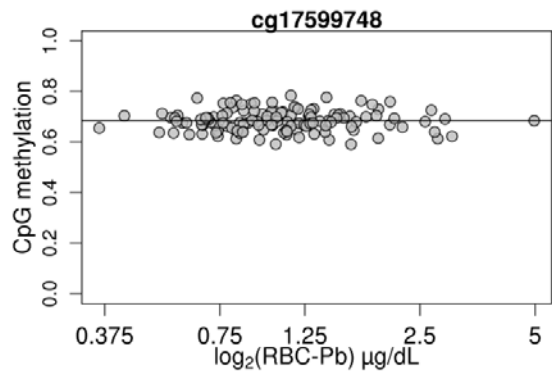
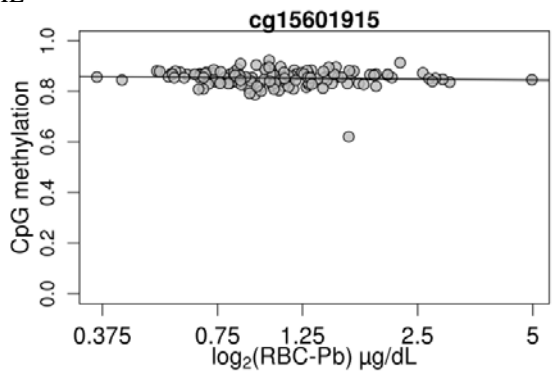




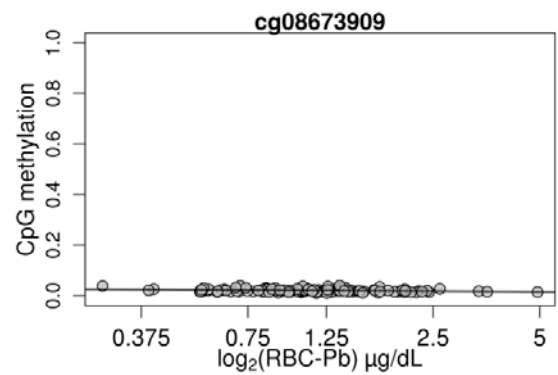
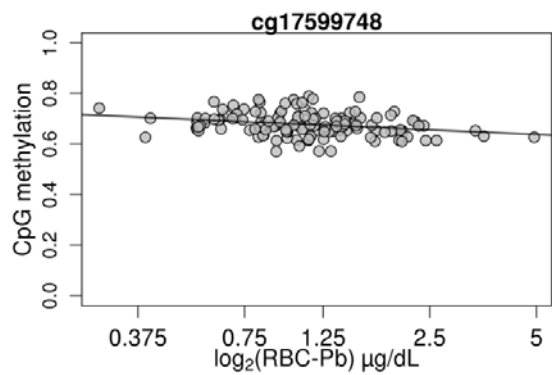
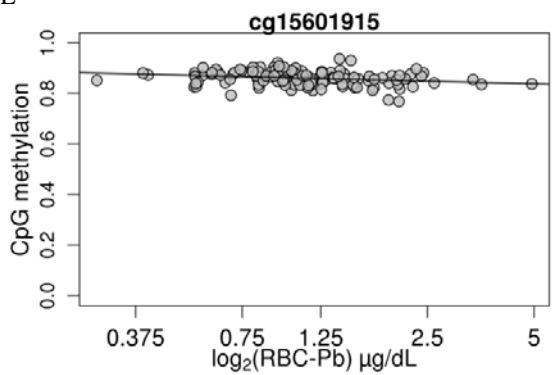
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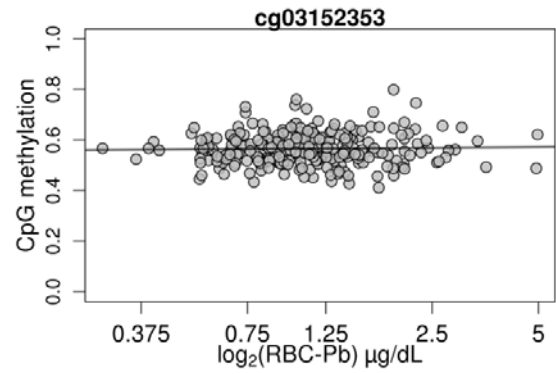
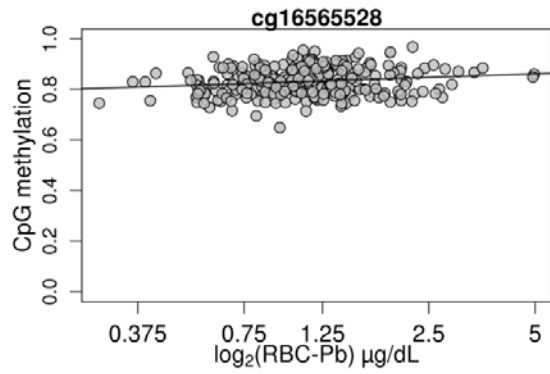
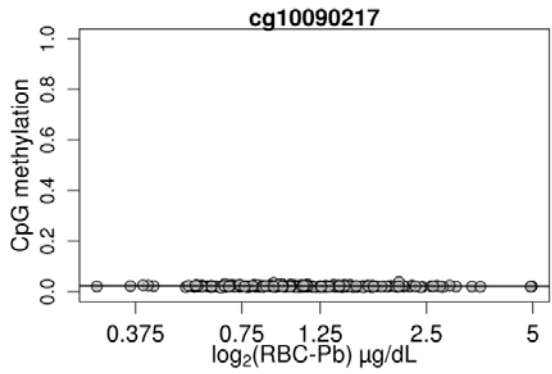
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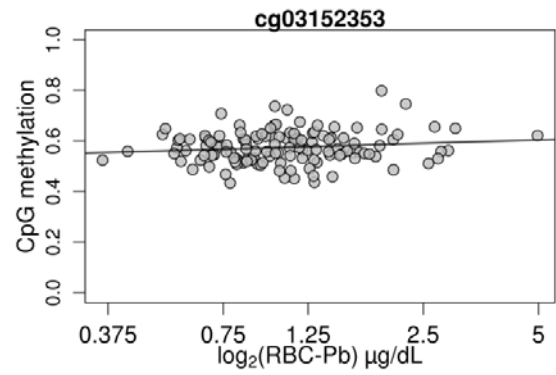
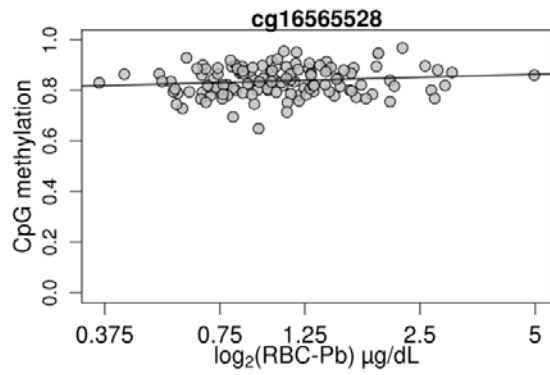
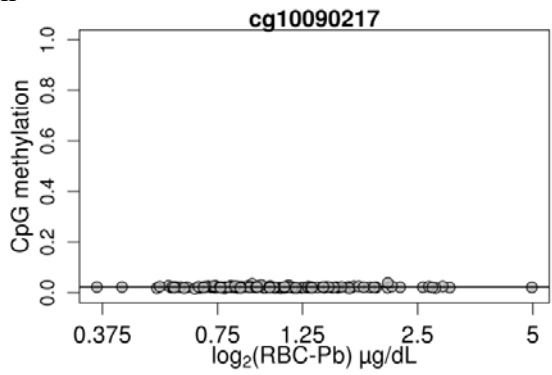
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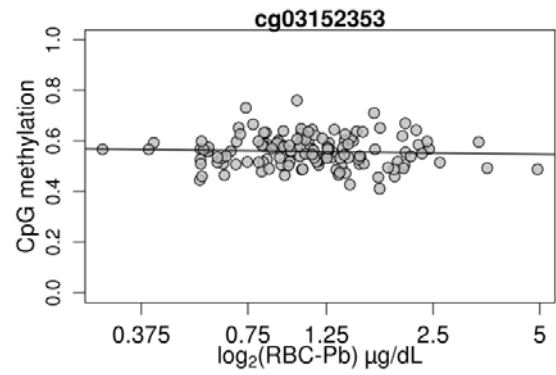
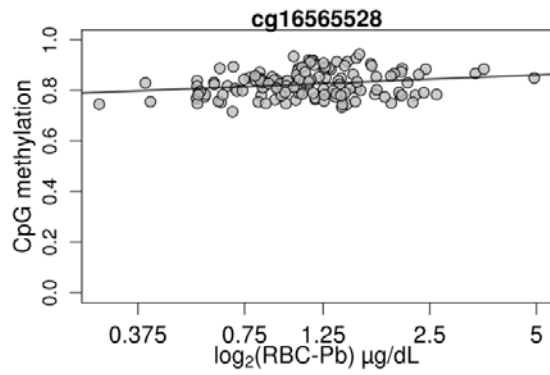
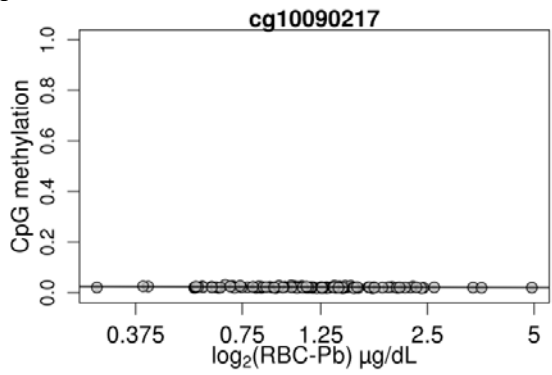
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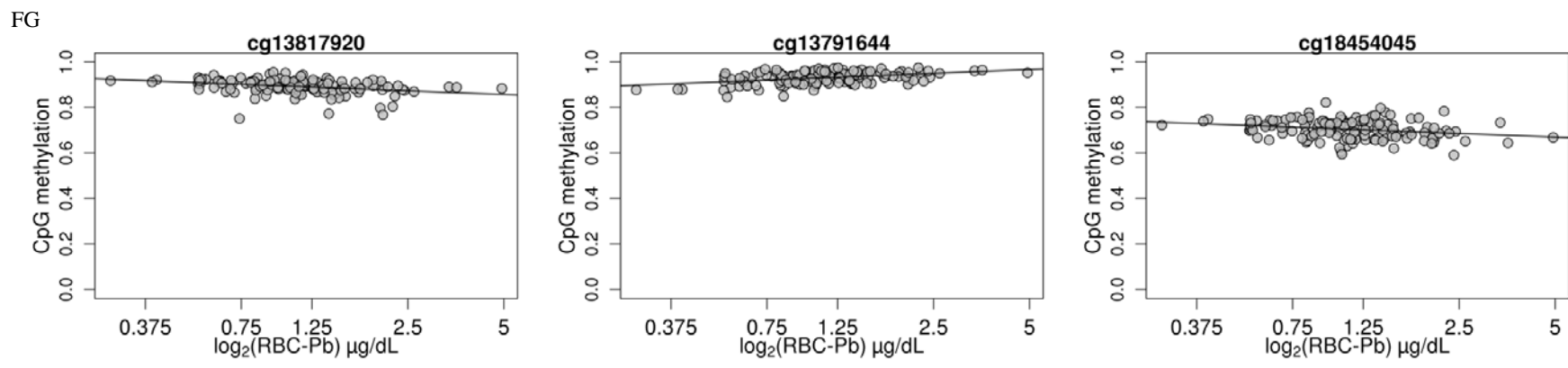
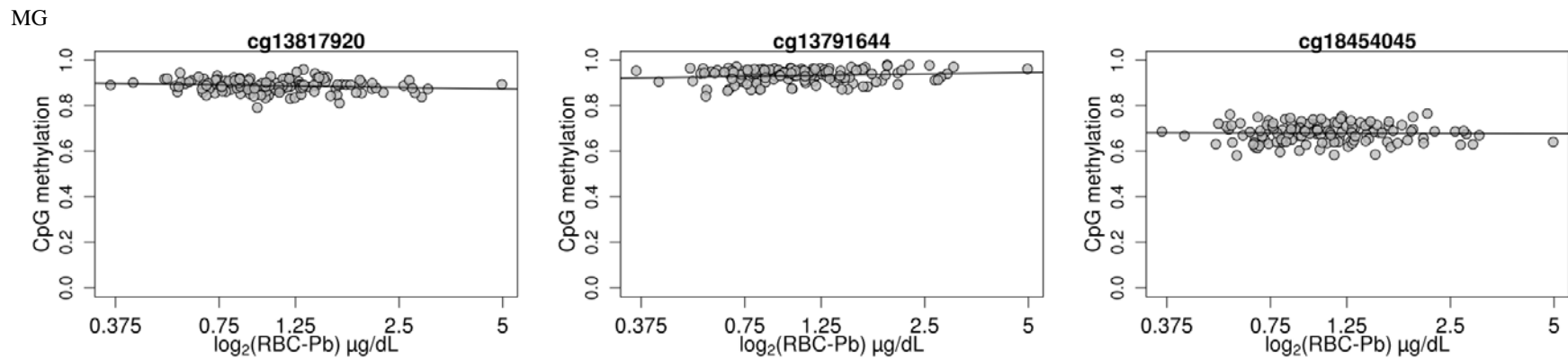
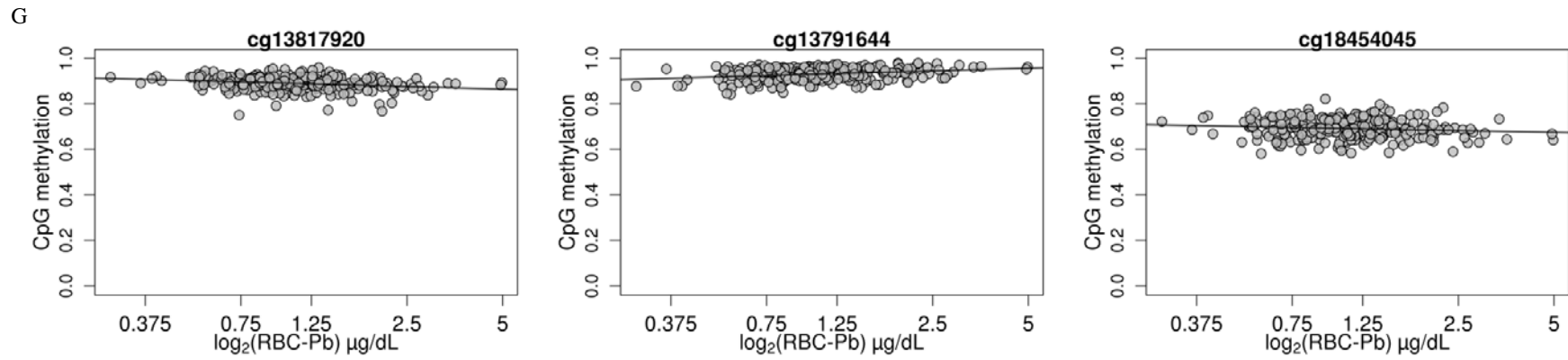


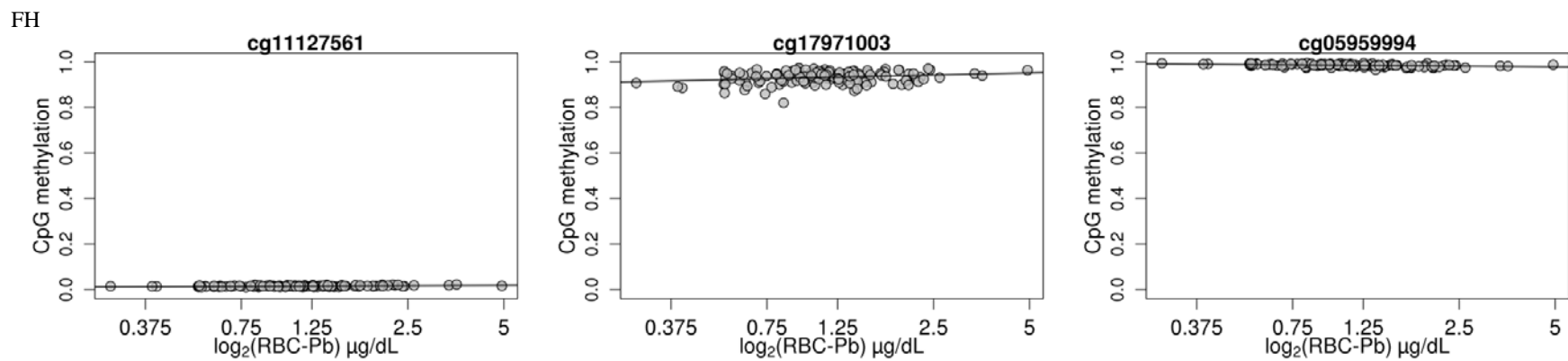
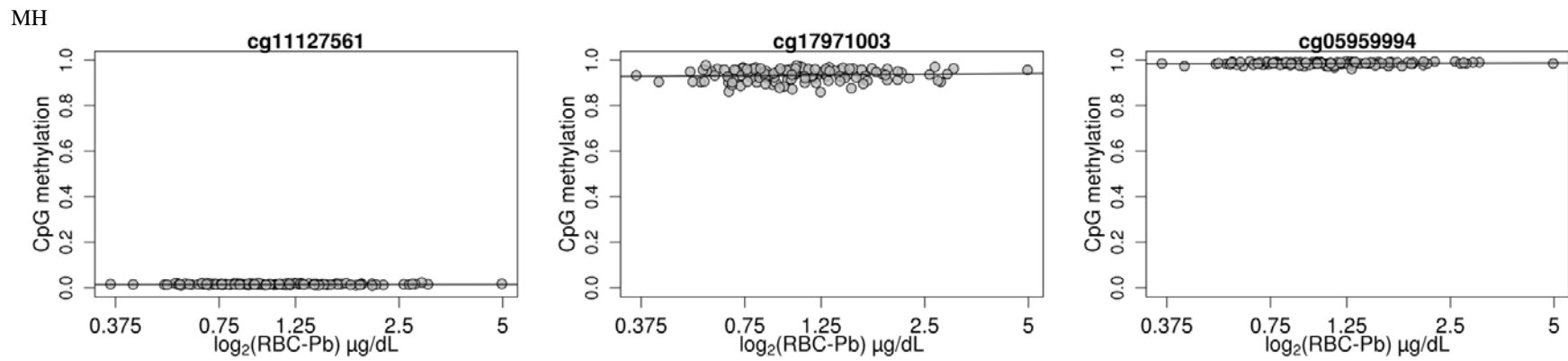
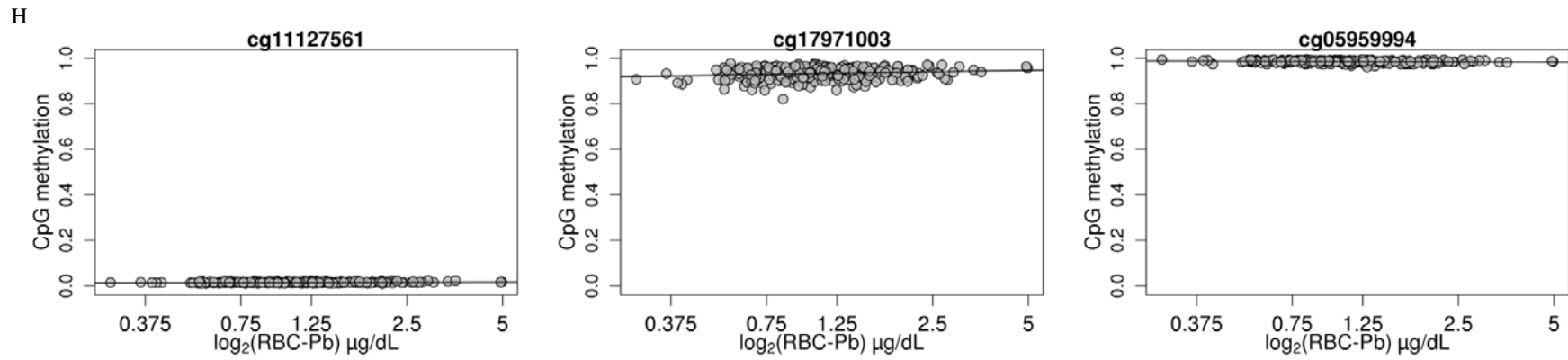
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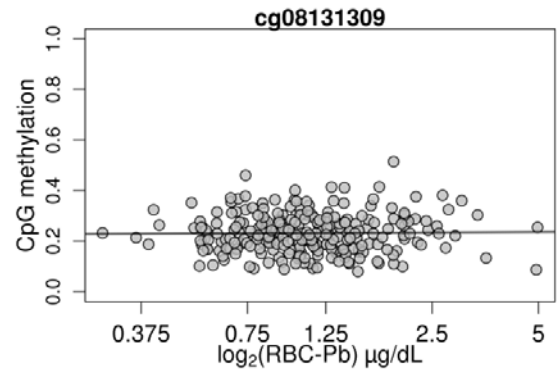
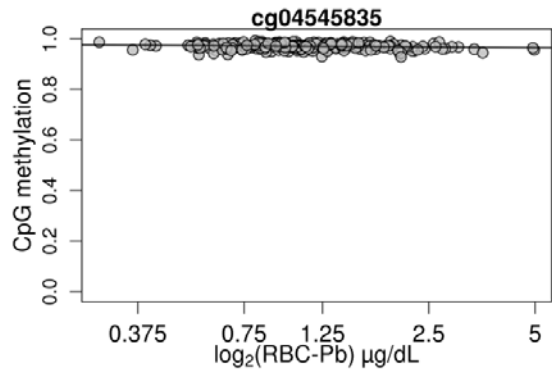
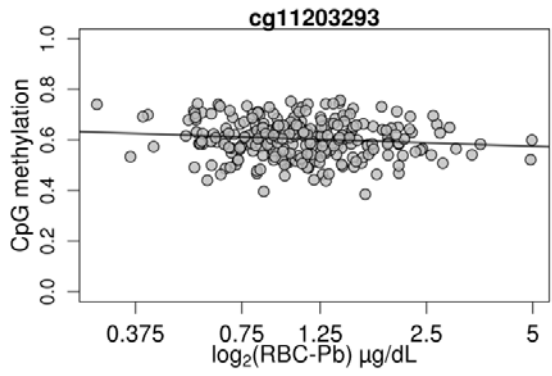
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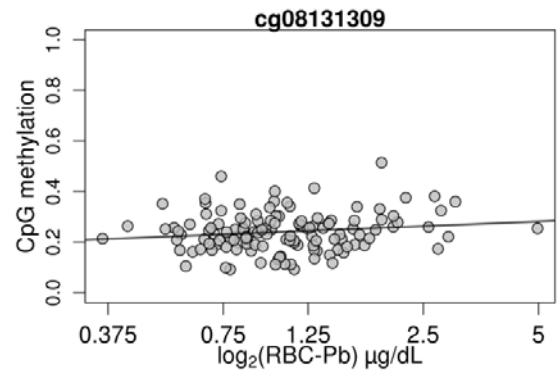
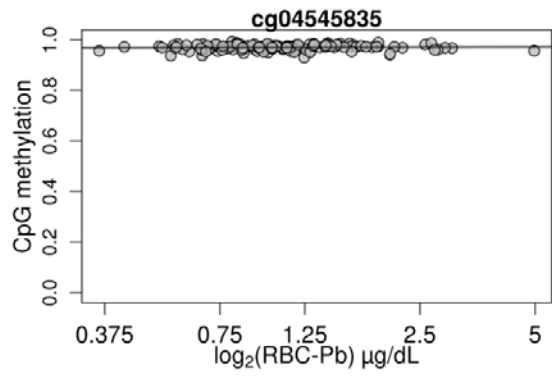
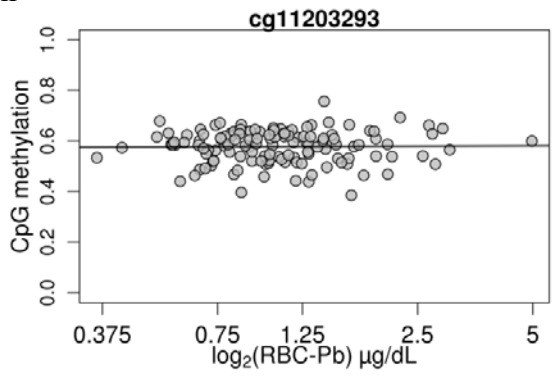




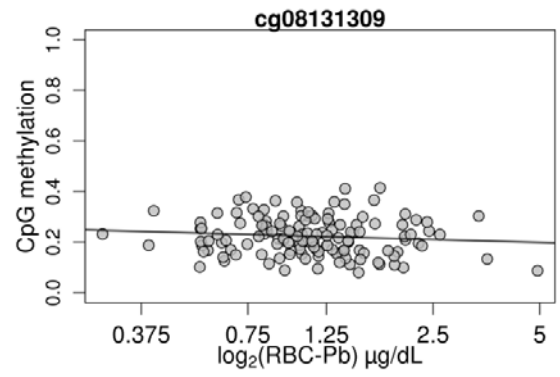
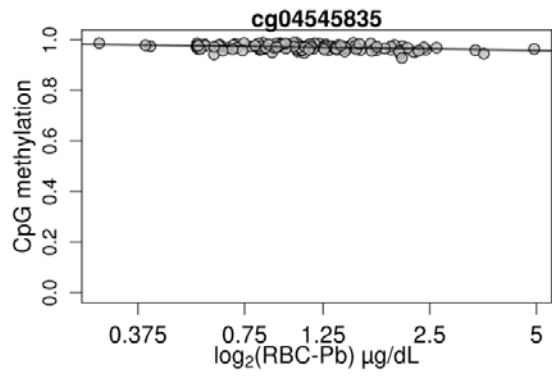
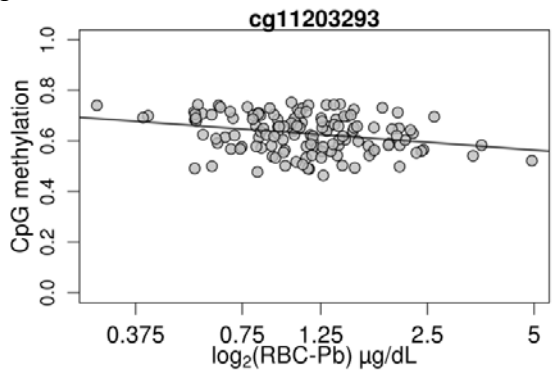
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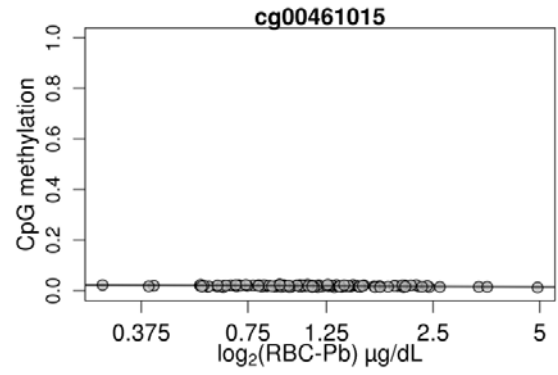
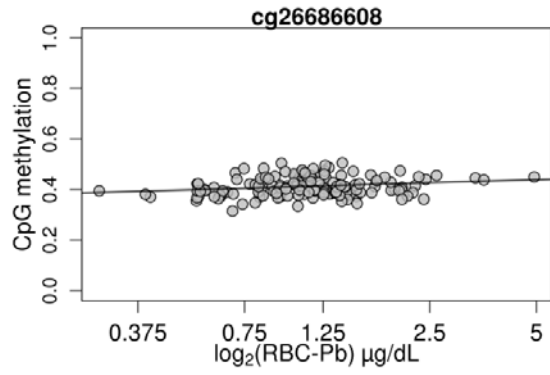
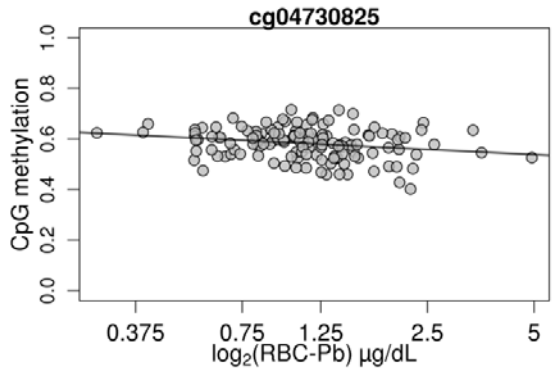
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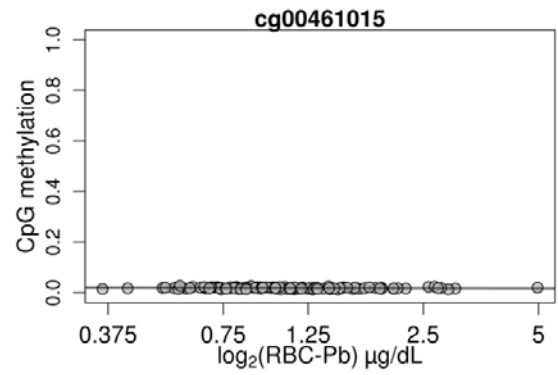
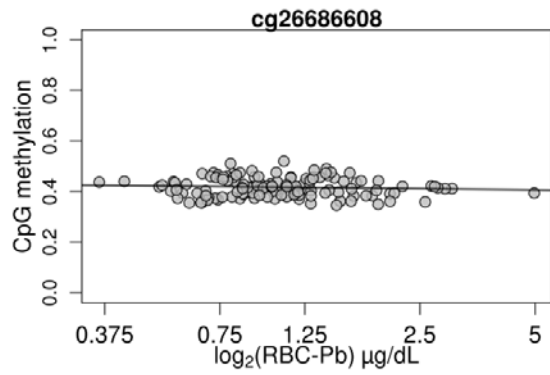
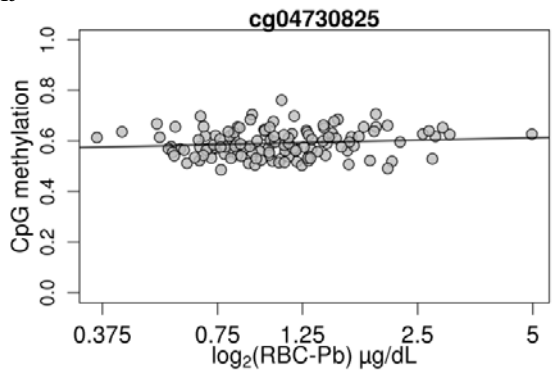
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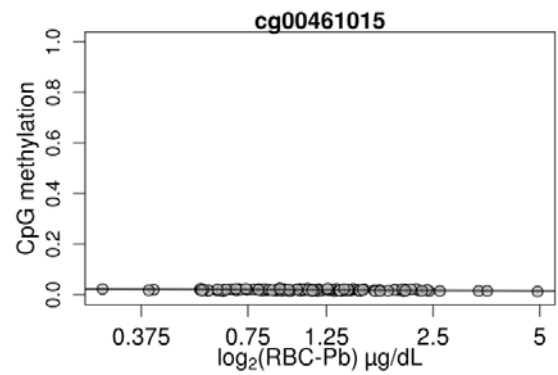
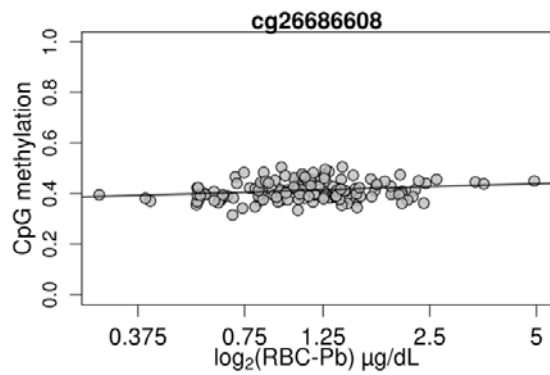
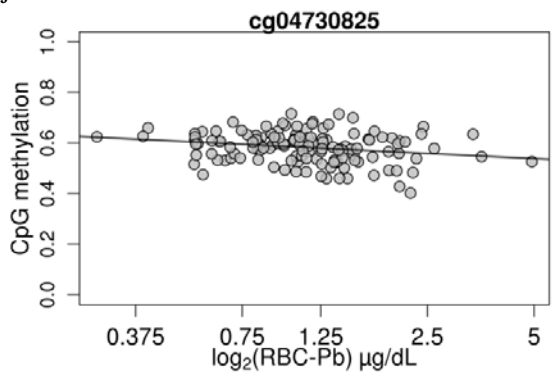
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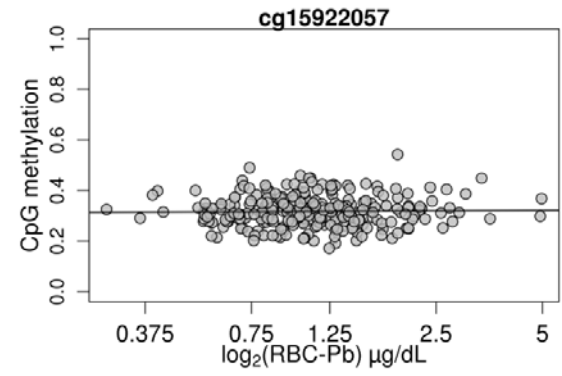
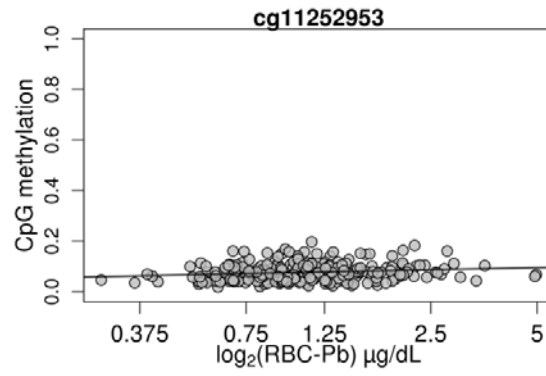
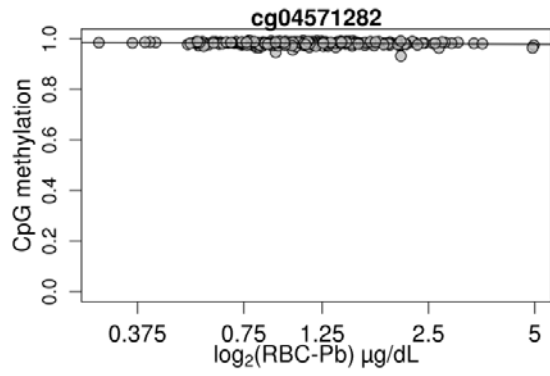
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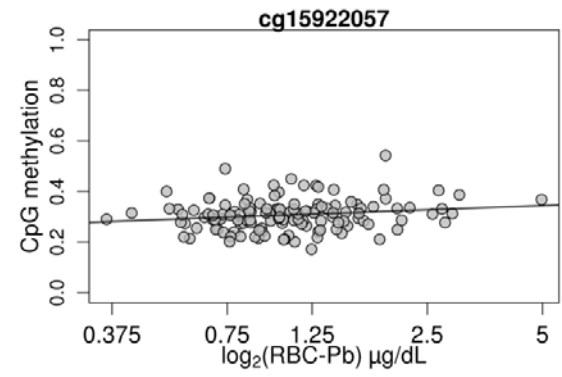
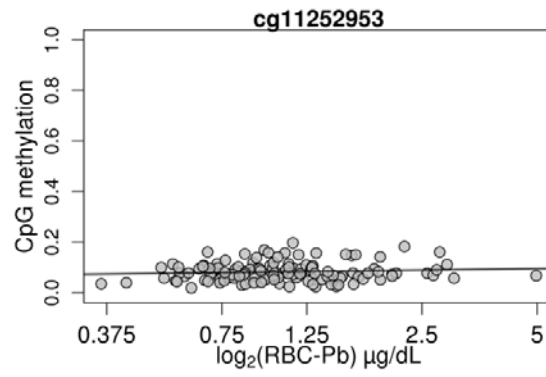
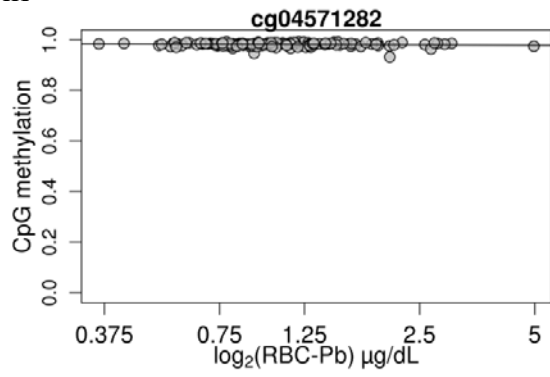
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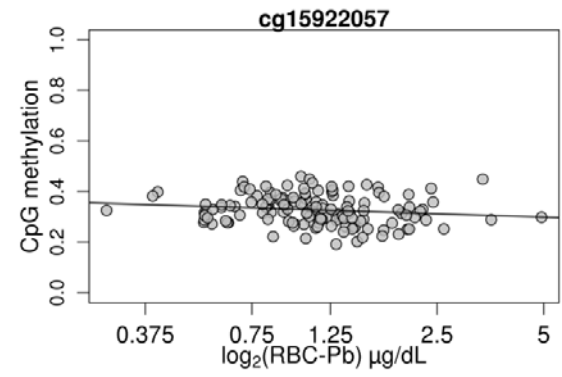
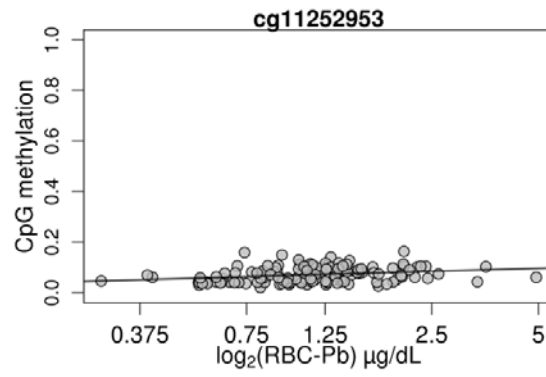
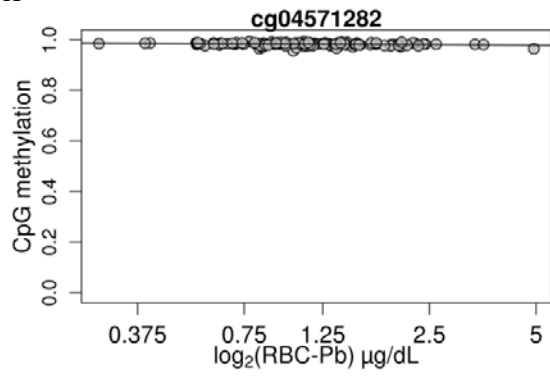
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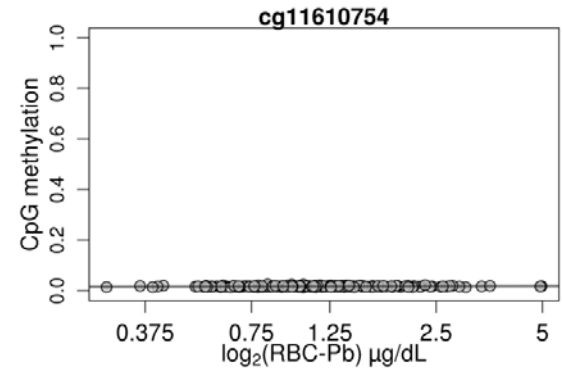
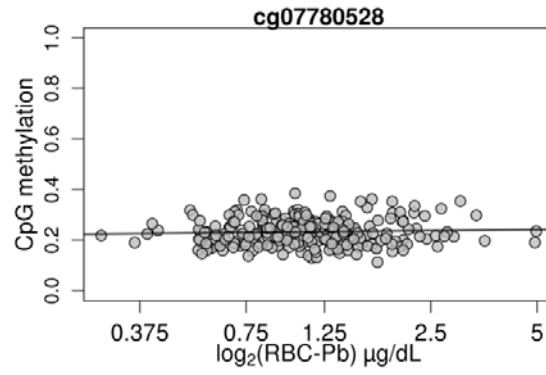
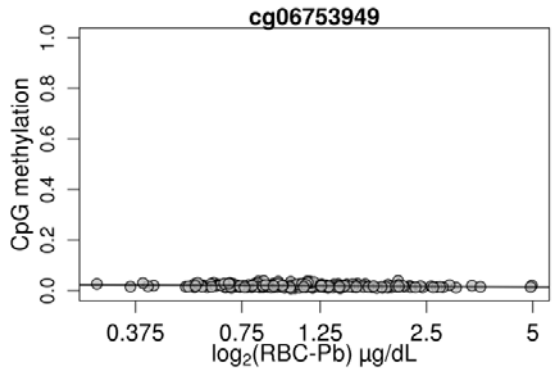


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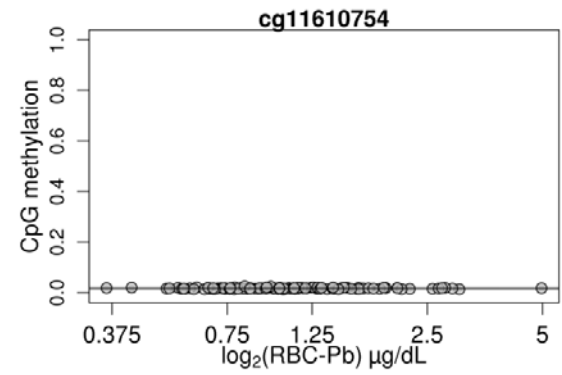
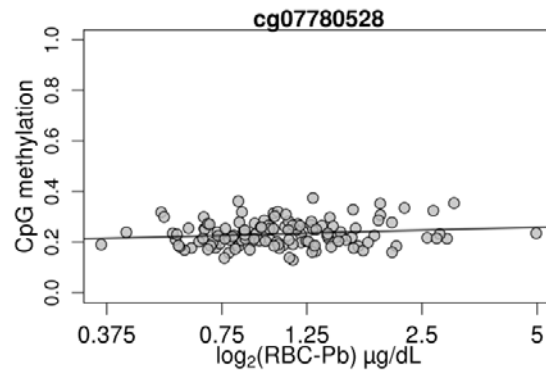
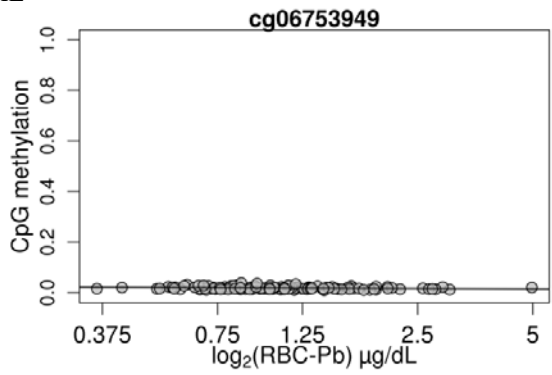




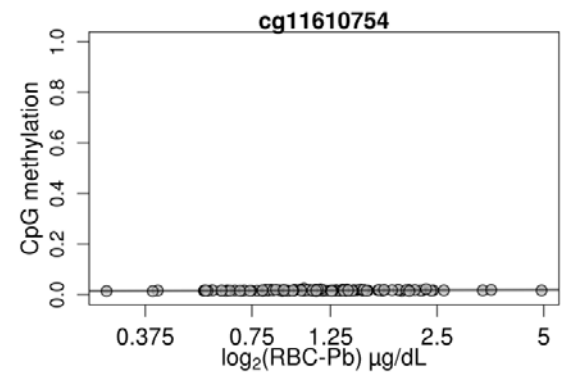
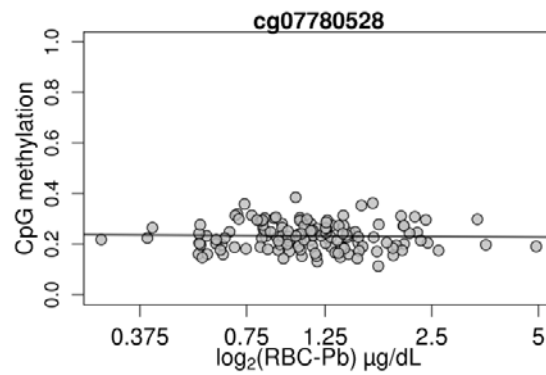
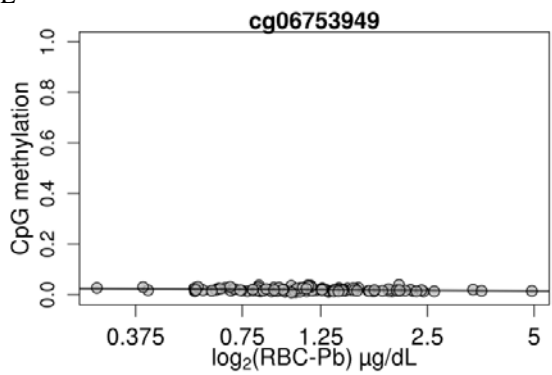
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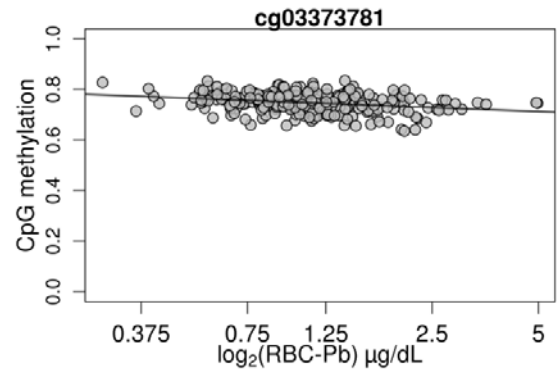
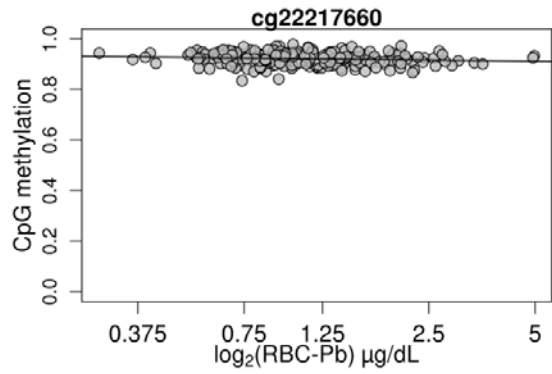
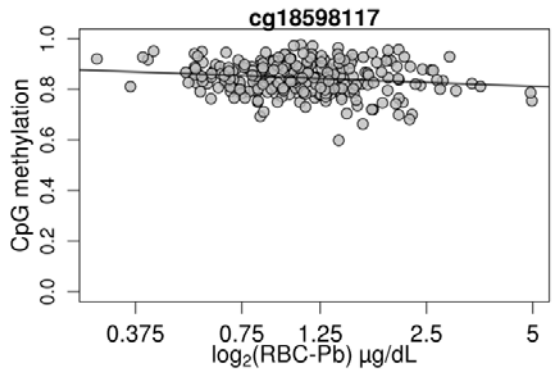
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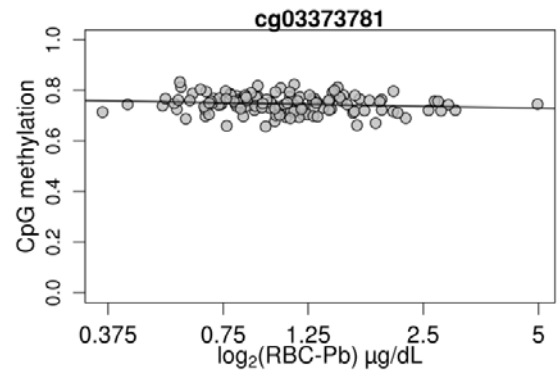
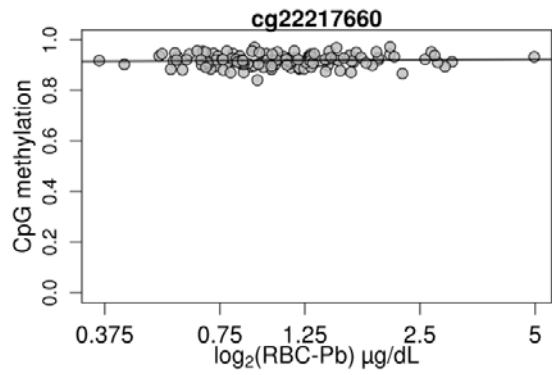
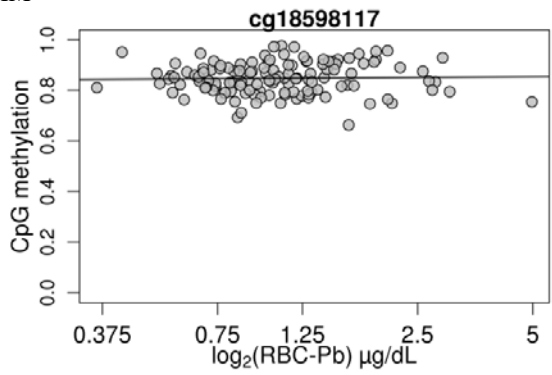
FL



M



MM



FM

