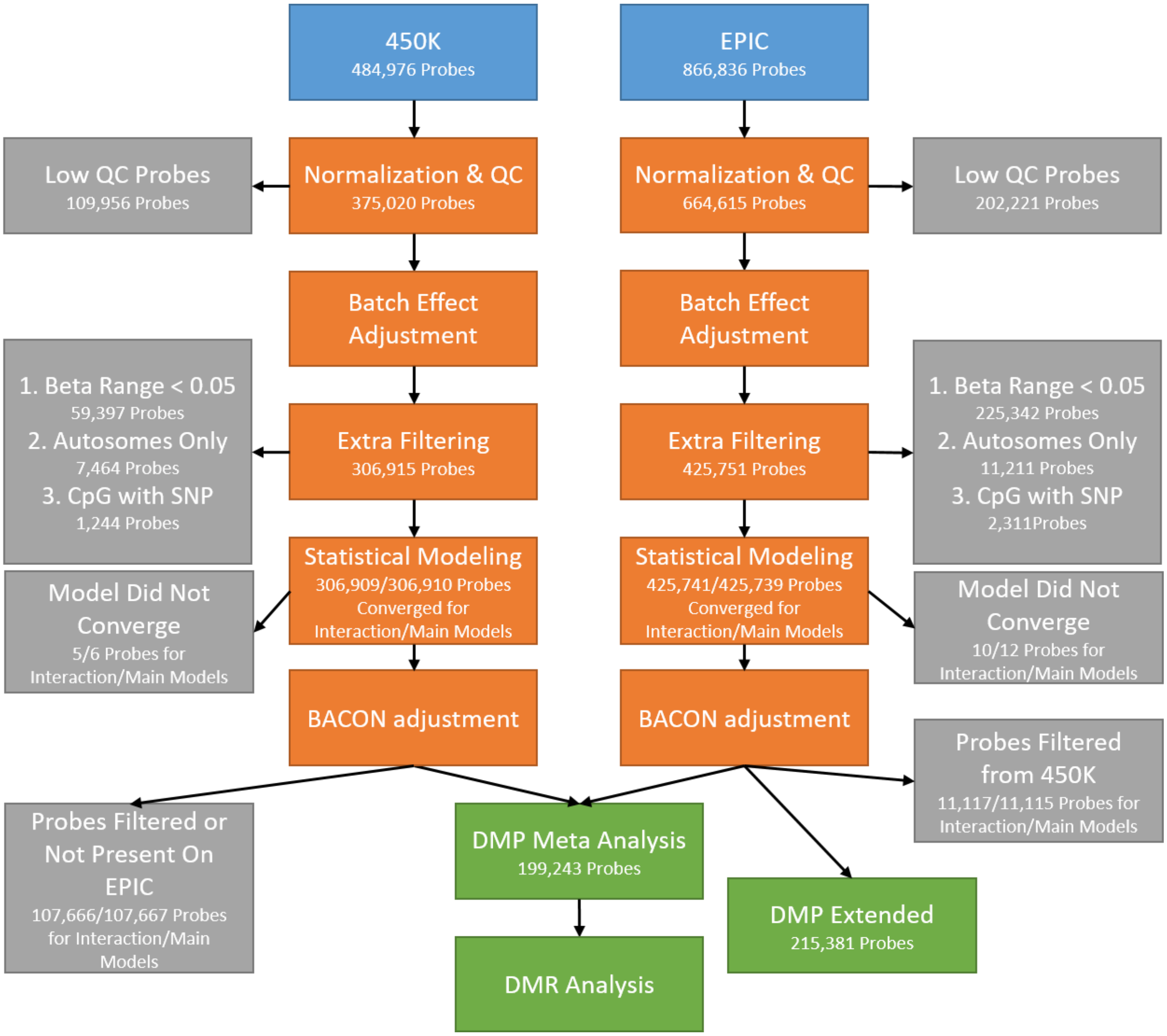
Longitudinal DNA methylation differences precede type 1 diabetes

Randi K Johnson, Lauren A Vanderlinden, Fran Dong, Patrick M Carry, Jennifer Seifert, Kathleen Waugh, Hanan Shorrosh, Tasha Fingerlin, Brigitte I. Frohnert, Ivana V Yang, Katerina Kechris, Marian Rewers, Jill M Norris

Supplementary Figure S1: Data pre-processing and quality control pipeline conducted in parallel on 450K and EPIC.

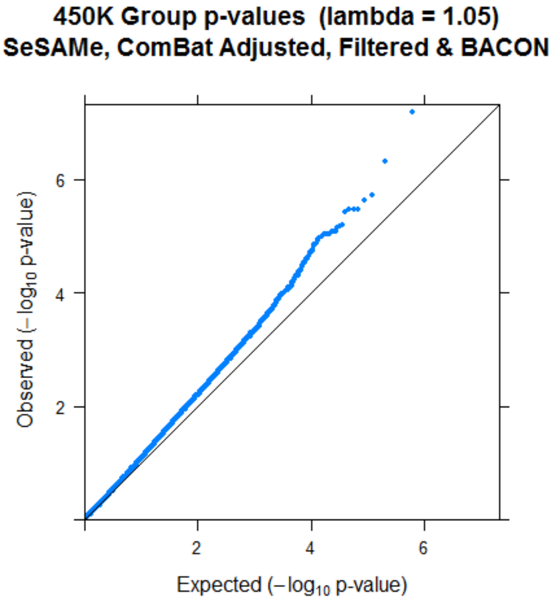


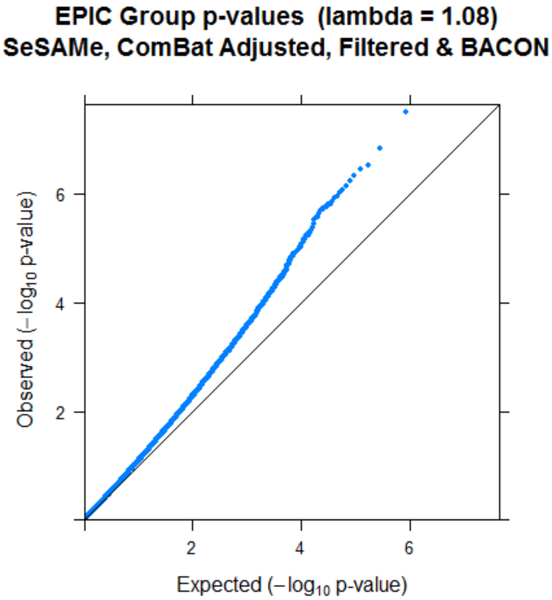
Supplementary Table S1: Differentially changing methylation regions (DCMRs) where the rate of methylation change with age differed between T1D cases and controls.

chr	start	end	n probes	slope control	slope case	adj pvalue	near gene	biotype*
chr1	68512650	68513064	7	0.0042	0.0020	0.00000672	DIRAS3	protein_coding
chr6	31734192	31734581	6	0.0100	0.0073	0.0000192	VWA7	protein_coding
chr1	223566447	223566795	6	0.0011	-0.0010	0.0002	C1orf65	protein_coding
chr14	24780404	24780735	6	-0.0018	-0.0045	0.000286	LTB4R, CIDEB	protein_coding
chr20	36148954	36149232	12	0.0015	-0.0004	0.0008869	NNAT	protein_coding
chr19	49223814	49224034	4	-0.0011	-0.0029	0.01516	RASIP1	protein_coding
chr16	83171068	83171300	3	-0.0077	-0.0050	0.01575	CTD-3253I12.1	Antisense
chr6	48036409	48036618	5	0.0019	0.0006	0.02318	PTCHD4	protein_coding
chr7	56515666	56515847	3	0.0009	-0.0009	0.02595	RP13-492C18.2	Pseudogene
chr19	36485282	36485361	3	0.0005	-0.0022	0.05705	SDHAF1	protein_coding

**Gene and transcript types defined by Ensembl annotation*

Supplementary Figure S2: QQplots and genomic inflation factors from average longitudinal differential methylation analyses on 450K and EPIC.


450K Group p-values (lambda = 1.05)
SeSAMe, ComBat Adjusted, Filtered & BACON

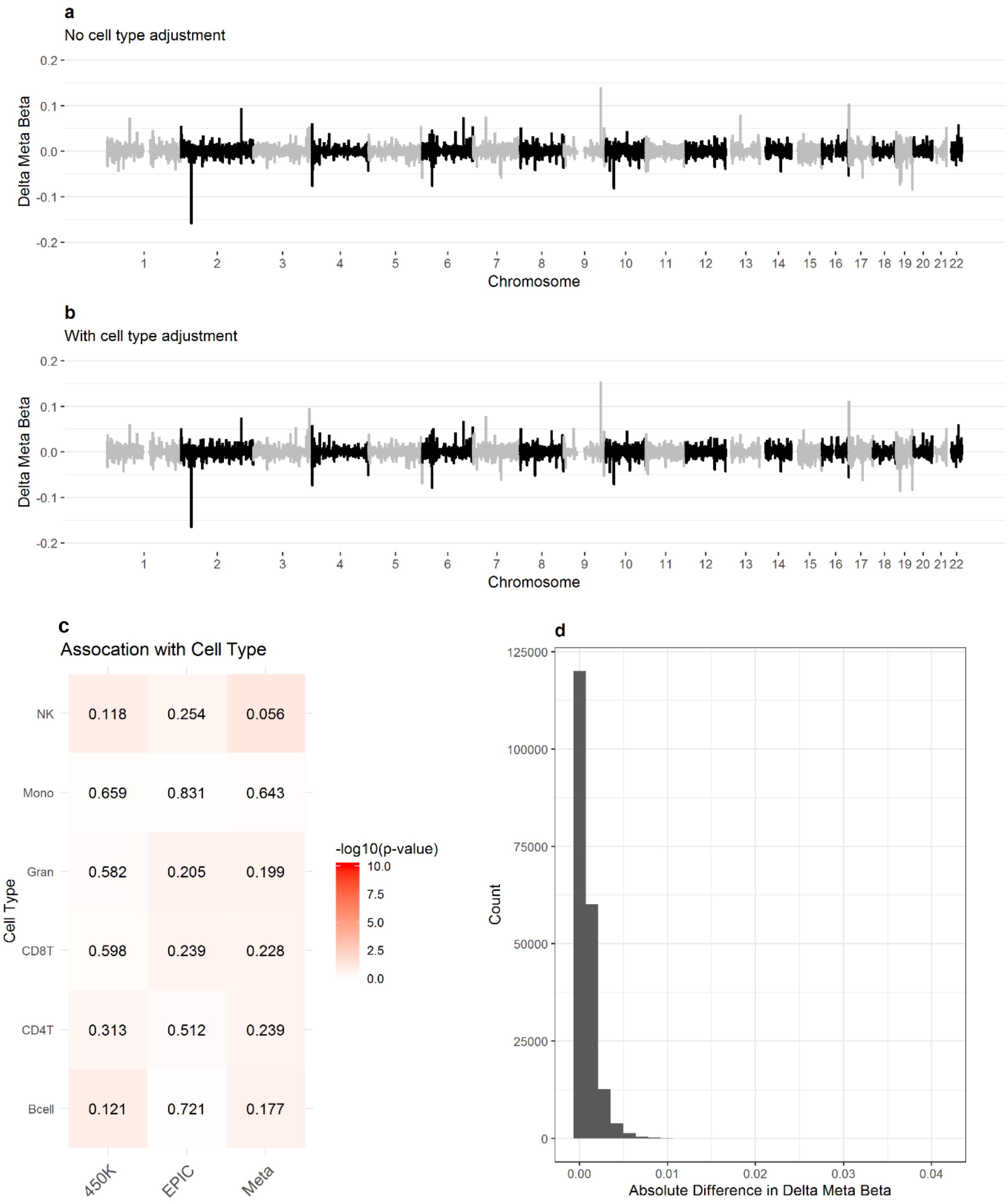

EPIC Group p-values (lambda = 1.08)
SeSAMe, ComBat Adjusted, Filtered & BACON

Supplementary Table S2: Top 10 enriched GO terms in 299 probes with meta p-value < 0.001.

Term	Ontology	Number of Genes	Number of Significant Genes	P-value	FDR	ID
isoquinoline alkaloid metabolic process	BP	2	2	0.000797	1	GO:0033076
phytoalexin metabolic process	BP	2	2	0.000797	1	GO:0052314
transport vesicle	CC	353	12	0.000834	1	GO:0030133
calcium-dependent cysteine-type endopeptidase activity	MF	15	3	0.001028	1	GO:0004198
calcium-dependent ATPase activity	MF	3	2	0.001072	1	GO:0030899
alkaloid metabolic process	BP	3	2	0.001181	1	GO:0009820
voltage-gated calcium channel complex	CC	42	5	0.001189	1	GO:0005891
troponin C binding	MF	3	2	0.001198	1	GO:0030172
oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, reduced pteridine as one donor, and incorporation of one atom of oxygen	MF	7	2	0.001357	1	GO:0016714
transport vesicle membrane	CC	188	8	0.0015	1	GO:0030658

Supplementary Table S3: Top 10 enriched KEGG pathways in 299 probes with meta p-value < 0.001.

Pathway	Number of Genes	Number of Significant Genes	P-value	FDR	ID
Tyrosine metabolism	31	3	0.006197	0.820089	path:hsa00350
Autoimmune thyroid disease	30	3	0.00725	0.820089	path:hsa05320
Type II diabetes mellitus	45	4	0.007455	0.820089	path:hsa04930
Type I diabetes mellitus	36	3	0.014499	1	path:hsa04940
Folate biosynthesis	25	2	0.017458	1	path:hsa00790
Dopaminergic synapse	127	5	0.033588	1	path:hsa04728
Cell adhesion molecules (CAMs)	128	5	0.035771	1	path:hsa04514
Rheumatoid arthritis	81	3	0.041011	1	path:hsa05323
Graft-versus-host disease	30	2	0.049472	1	path:hsa05332
Allograft rejection	29	2	0.050154	1	path:hsa05330



Supplementary Figure S3: Sensitivity analysis adjusting for cell type proportions in longitudinal meta-analysis for 199,230 probes. a-b) Delta meta beta (methylation difference) between T1D cases and controls with and without cell type adjustment. c) P-value heat map of longitudinal associations of cell types with T1D from linear mixed models adjusted for sex and age, by panel and meta-analysis. d) Absolute difference (cell type adjusted – non-adjusted) in delta meta beta (median = 5.4 X 10^{-4}, IQR: 2.4 X 10^{-4} to 1.1 X 10^{-3}).

Supplementary Table S4: Differentially methylated regions associated with development of T1D.

DMR	chr	start	end	n probes	pct hyper	avg beta	adj pvalue	near gene	biotype
1	chr9	124989241	124990457	7	100	0.071259405	2.04E-10	LHX6	protein_coding
2	chr4	57547347	57548094	5	100	0.032198331	6.4E-09	HOPX	protein_coding
3	chr12	96350519	96350796	5	0	-0.021779598	0.000000587	AMDHD1	protein_coding
4	chr17	47287410	47287578	4	100	0.012087365	0.00000284	ABI3	protein_coding
5	chr8	1113291	1113433	2	100	0.03731453	0.0000198	ERICH1-AS1	antisense
6	chr2	43903227	43903651	4	0	-0.013392819	0.0000402	AC011242.6	pseudogene
7	chr2	177014849	177015126	6	0	-0.013086321	0.000095	MIR10B	miRNA
8	chr12	1725788	1726077	5	0	-0.012564324	0.0001327	FBXL14	protein_coding
9	chr8	1649868	1650173	3	100	0.026054591	0.0002919	DLGAP2	protein_coding
10	chr8	1012324	1012466	2	100	0.013360668	0.0005535	CTD-2281E23.2	processed_transcript
11	chr6	28973328	28973521	6	0	-0.004953171	0.001018	ZNF311	protein_coding
12	chr2	202901352	202901471	3	100	0.070727636	0.002206	FZD7	protein_coding
13	chr8	1273604	1273857	4	100	0.021114917	0.005035	CTD-2281E23.1	lincRNA
14	chr22	31002892	31003148	7	0	-0.003539409	0.005239	TCN2, PES1	protein_coding
15	chr7	94953810	94954203	5	0	-0.041959065	0.006206	AC004022.7	pseudogene
16	chr17	1395864	1396124	5	100	0.016914269	0.007037	MYO1C	protein_coding
17	chr2	1452260	1452368	2	100	0.018378147	0.00868	TPO	protein_coding
18	chr18	19756877	19757023	3	0	-0.005802175	0.01757	RP11-627G18.2	antisense
19	chr10	3466795	3466853	2	100	0.028986776	0.0195	RP11-482E14.1	lincRNA
20	chr16	1060367	1060559	3	100	0.015585702	0.02279	RP11-161M6.3	lincRNA
21	chr19	35800589	35800744	2	0	-0.027056551	0.02335	MAG	protein_coding
22	chr7	12443880	12444116	5	0	-0.027820051	0.03191	VWDE	protein_coding
23	chr13	111956623	111956696	2	100	0.013535115	0.04095	ARHGEF7	protein_coding
24	chr17	45924888	45925061	2	0	-0.022103851	0.04459	SP6	protein_coding
25	chr7	73157217	73157384	2	100	0.036716466	0.0455	ABHD11	protein_coding
26	chr20	17296317	17296451	2	0	-0.011337132	0.04911	PCSK2	protein_coding
27	chr17	42733527	42733699	5	0	-0.011711252	0.07489	C17orf104	protein_coding

28	chr16	89164953	89164991	2	0	-0.011399305	0.09162	ACSF3	protein_coding

Supplementary Table S5: Longitudinal and cross-sectional results for candidate probes associated with the development of T1D (DMPs or within DMRs).

probe	type	chr	pos	Long FDR	Long pvalue	PreSV pvalue	PostSV pvalue	CB pvalue	Long Beta	PreSV Beta	PostSV Beta	CB Beta	near gene	biotype
cg25705717	DMP	chr10	27608719	3.92E-03	1.97E-08	2.24E-04	2.58E-06	2.23E-01	-0.081	-0.076	-0.079	-0.045	RNU6-666P	snRNA
cg19309499	DMP	chr8	1150488	8.89E-02	8.92E-07	3.62E-04	4.22E-05	2.17E-01	0.025	0.026	0.023	0.007	CTD-2281E23.3	lincRNA
cg03970350	DMR	chr22	31003010	1.99E-01	5.00E-06	5.81E-02	7.90E-07	3.26E-01	-0.006	-0.005	-0.008	-0.003	PES1	protein_coding
cg06874426	DMR	chr17	47287526	1.99E-01	4.11E-06	4.74E-03	3.49E-04	2.46E-02	0.013	0.012	0.013	0.007	ABI3	protein_coding
cg19530281	DMR	chr8	1113432	2.24E-01	6.74E-06	1.36E-04	7.53E-04	2.54E-03	0.034	0.036	0.028	0.026	ERICH1-AS1	antisense
cg16407924	DMR	chr2	1452260	2.52E-01	1.07E-05	4.04E-03	1.89E-05	6.54E-02	0.023	0.018	0.024	0.008	TPO	protein_coding
cg04085076	DMR	chr4	57547579	2.73E-01	3.61E-05	2.59E-03	3.66E-05	1.65E-06	0.034	0.030	0.035	0.050	HOPX	protein_coding
cg26410635	DMR	chr12	96350720	2.73E-01	2.73E-05	5.31E-03	2.45E-05	2.82E-02	-0.033	-0.033	-0.036	-0.037	AMDHD1	protein_coding
cg27509052	DMR	chr8	1012324	2.73E-01	3.81E-05	8.47E-04	2.10E-04	6.87E-01	0.022	0.024	0.020	-0.005	CTD-2281E23.2	processed_transcript
cg20373635	DMR	chr10	3466795	2.75E-01	4.37E-05	2.43E-02	4.41E-06	4.51E-04	0.030	0.021	0.036	0.034	RP11-482E14.1	lincRNA
cg27351978	DMR	chr8	1650172	2.80E-01	5.48E-05	2.06E-04	6.90E-04	2.23E-01	0.046	0.058	0.039	0.011	DLGAP2	protein_coding
cg10531725	DMR	chr17	45924888	3.68E-01	8.77E-05	1.21E-01	6.71E-05	4.23E-01	-0.031	-0.020	-0.033	-0.022	SP6	protein_coding
cg17104824	DMR	chr2	177014959	3.68E-01	8.27E-05	2.94E-01	1.97E-03	1.23E-01	-0.017	-0.007	-0.016	-0.015	MIR10B	miRNA
cg05104581	DMR	chr16	89164990	4.68E-01	1.64E-04	2.04E-04	8.44E-03	5.24E-03	-0.013	-0.020	-0.010	-0.020	ACSF3	protein_coding
cg09132607	DMR	chr2	43903582	4.68E-01	1.71E-04	2.07E-01	3.14E-03	1.53E-01	-0.011	-0.006	-0.010	-0.005	AC011242.6	pseudogene
cg10696062	DMR	chr12	1726028	4.68E-01	1.71E-04	1.42E-02	2.59E-03	8.09E-01	-0.019	-0.022	-0.018	-0.001	FBXL14	protein_coding
cg25674613	DMR	chr8	1273808	5.06E-01	2.31E-04	4.29E-02	2.08E-03	4.11E-01	0.028	0.019	0.026	-0.002	CTD-2281E23.1	lincRNA
cg00142257	DMR	chr9	124990276	5.46E-01	3.32E-04	1.46E-01	7.09E-05	2.91E-01	0.072	0.039	0.083	0.017	LHX6	protein_coding

cg10435235	DMR	chr13	111956623	5.82E-01	4.81E-04	6.22E-03	3.10E-02	4.23E-01	0.014	0.017	0.010	-0.011	ARHGEF7	protein_coding
cg26127652	DMR	chr6	28973426	5.82E-01	5.06E-04	9.53E-02	4.11E-03	6.54E-01	-0.005	-0.005	-0.005	-0.002	ZNF311	protein_coding
cg09165842	DMR	chr20	17296317	5.86E-01	5.18E-04	8.95E-02	4.77E-03	1.71E-01	-0.011	-0.009	-0.010	-0.013	PCSK2	protein_coding
cg27638615	DMR	chr2	202901352	6.07E-01	6.39E-04	2.53E-01	1.38E-03	1.74E-01	0.062	0.028	0.062	0.016	FZD7	protein_coding
cg12615165	DMR	chr18	19756877	6.14E-01	7.23E-04	4.17E-04	5.46E-03	3.69E-02	-0.004	-0.008	-0.005	-0.004	RP11-627G18.2	antisense
cg22238209	DMR	chr19	35800743	6.14E-01	7.15E-04	6.88E-04	5.21E-04	5.88E-01	-0.037	-0.073	-0.044	-0.033	MAG	protein_coding
cg26010879	DMR	chr7	73157217	6.72E-01	1.15E-03	8.63E-02	6.58E-04	1.21E-03	0.033	0.026	0.035	0.034	ABHD11	protein_coding
cg07091798	DMR	chr16	1060367	7.09E-01	2.15E-03	9.49E-03	6.28E-03	3.89E-02	0.015	0.015	0.014	0.013	RP11-161M6.3	lincRNA
cg00597076	DMR	chr17	1395880	7.15E-01	2.28E-03	2.29E-01	4.70E-03	7.70E-01	0.016	0.009	0.016	-0.002	MYO1C	protein_coding
cg17330251	DMR	chr7	94953956	7.38E-01	3.00E-03	3.31E-01	9.72E-03	6.18E-01	-0.058	-0.041	-0.054	-0.017	AC004022.7	pseudogene
cg03579179	DMR	chr7	12444095	7.87E-01	6.55E-03	3.13E-03	1.09E-01	8.35E-01	-0.034	-0.072	-0.022	-0.021	VWDE	protein_coding
cg15000379	DMR	chr17	42733662	7.95E-01	7.05E-03	3.90E-02	9.79E-02	7.08E-01	-0.006	-0.010	-0.004	-0.001	C17orf104	protein_coding

Supplementary Methods: Multivariable regression equations

Differentially changing methylation positions (DCMPs)

$$DNA\ methylation_{ij} = \beta_0 + \beta_{case} * caseStatus_{ij} + \beta_{age} * age_{ij} + \beta_{case*age} * caseStatus_{ij} * age_{ij} + \beta_{sex} * sex_{ij} + \varepsilon_{ij}$$

Where:

- $DNA\ methylation_{ij}$ is the M-value DNA methylation for the j^{th} of n_i observations in the i^{th} person
- $\beta_0, \ldots . \beta_{case}$ are the fixed-effect coefficients (including the intercept), which is identical for all subjects
- $caseStatus_{ij}$ is the fixed-effect case status variable (either case or control) for observation *j* in subject *i*
- age_{ij} is the fixed-effect age variable (measured out to reflect the specific day) for observation *j* in subject *i*
- $caseStatus_{ij} * age_{ij}$ is the fixed-effect interaction between the case status and age variables for observation *j* in subject *i*
- sex_{ij} is the fixed-effect sex variable (either male or female) for observation *j* in subject *i*
- $\varepsilon_{ij} = \phi\varepsilon_{ij-1} + Z_{ij}, \quad Z_{ij} \sim iid\ N(0, \sigma_Z^2)$ where ε_{ij} is the error for observation *j* in subject *i* and ϕ is the autocorrelation coefficient (fitting an autoregressive order 1 covariance structure)

Differential methylation positions (DMPs)

$$DNA\ methylation_{ij} = \beta_0 + \beta_{case} * caseStatus_{ij} + \beta_{age} * age_{ij} + \beta_{sex} * sex_{ij} + \varepsilon_{ij}$$

Where:

- $DNA\ methylation_{ij}$ is the M-value DNA methylation for the j^{th} of n_i observations in the i^{th} person
- $\beta_0, \ldots . \beta_{case}$ are the fixed-effect coefficients (including the intercept), which is identical for all subjects
- $caseStatus_{ij}$ is the fixed-effect case status variable (either case or control) for observation *j* in subject *i*
- age_{ij} is the fixed-effect age variable (measured out to reflect the specific day) for observation *j* in subject *i*
- sex_{ij} is the fixed-effect sex variable (either male or female) for observation *j* in subject *i*
- $\varepsilon_{ij} = \phi\varepsilon_{ij-1} + Z_{ij}, \quad Z_{ij} \sim iid\ N(0, \sigma_Z^2)$ where ε_{ij} is the error for observation *j* in subject *i* and ϕ is the autocorrelation coefficient (fitting an autoregressive order 1 covariance structure)