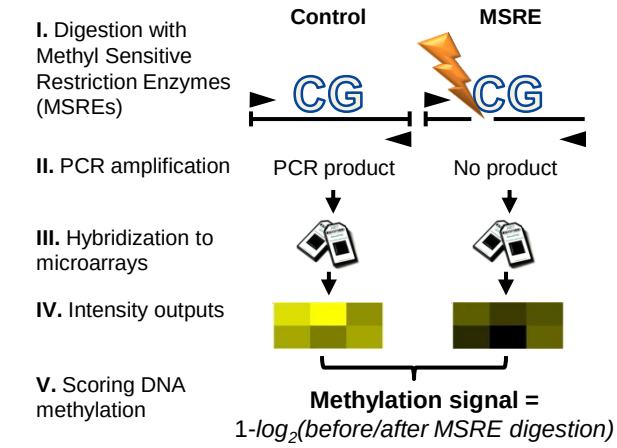
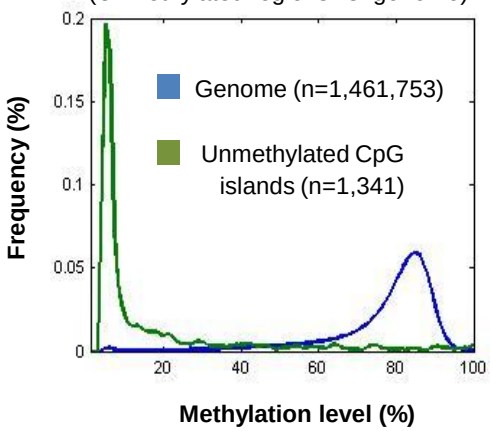
a **Microarray-based methylation assay**



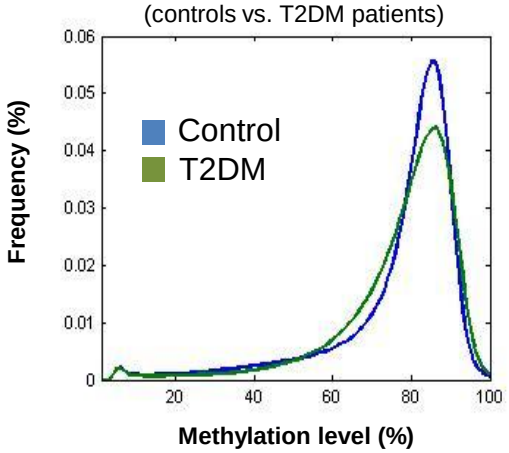
b **Methyl Sensitive Restriction Enzymes (MSREs)**

MSRE	Recognition sequence
Aci I	C**CG**C
BsaH I	GR**CG**YC
Hha I	G**CG**C
Hpa II	C**CG**G
HpyCH4 IV	A**CG**T

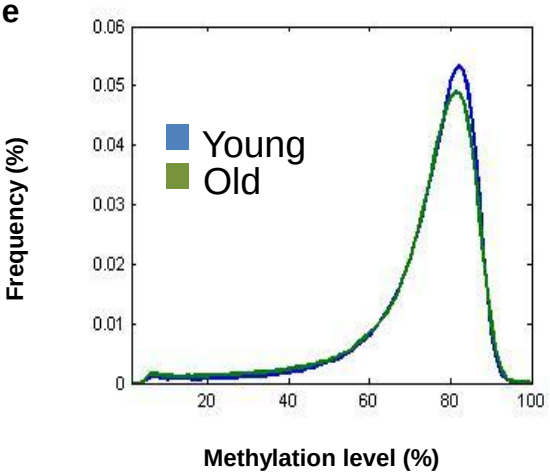
c **Distributions of methylation levels**
(Unmethylated regions vs. genome)



d **Distributions of methylation levels**
(controls vs. T2DM patients)



e **Distributions of methylation levels**
(Young vs. old controls)



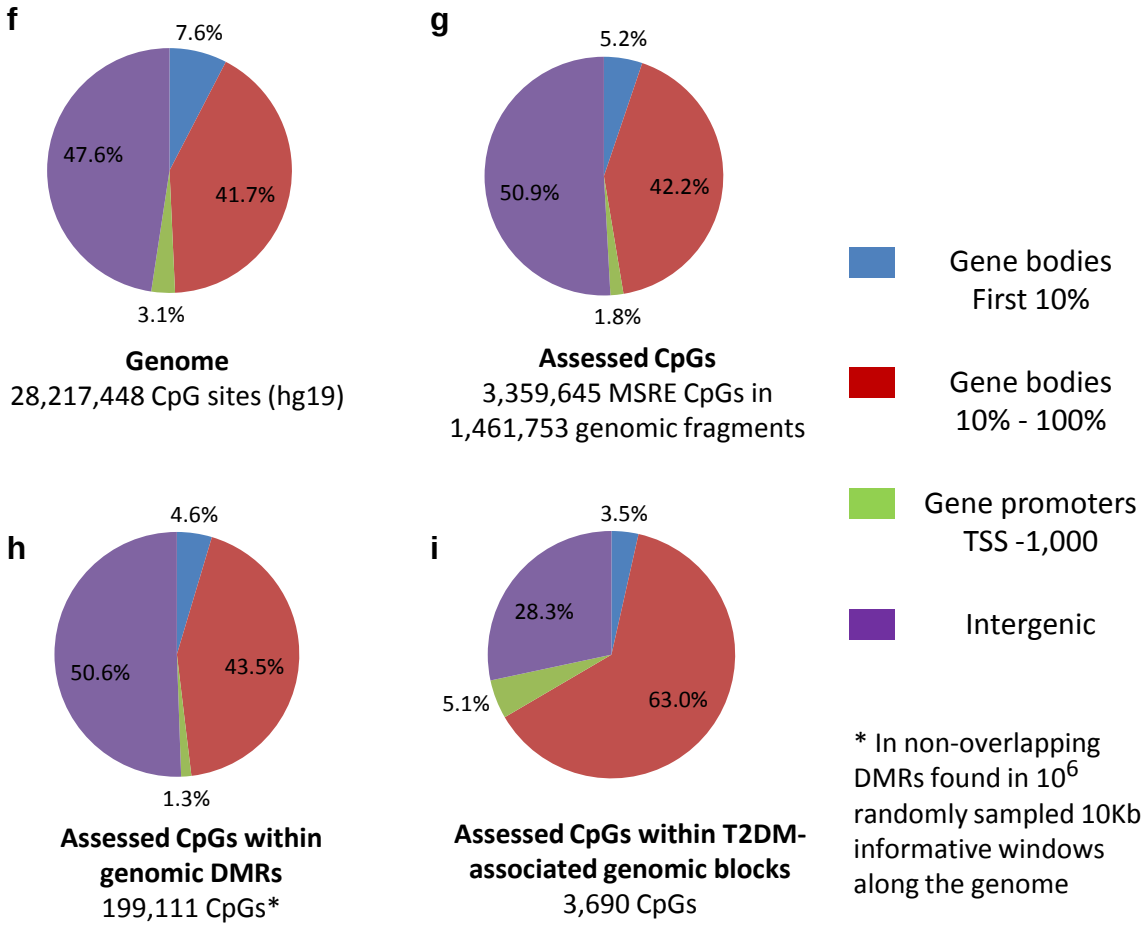


Figure S1. **Microarray-based methylation assessment. a.** A flow chart of the assay: genomic DNA samples were treated with a cocktail of five methyl sensitive restriction enzymes (MSREs), leading to digestion of unmethylated MSRE sites, while methylated sites remained intact (I). Treated and untreated DNAs were then PCR amplified from universal primers (arrowheads) (II) , and the resulting 200-1,100bp genomic fragments containing the MSRE sites were labeled and hybridized to mapping microarrays (III). This procedure led to a smaller amount of PCR product and consequently to reduced hybridization intensities of fragments enclosing unmethylated MSRE sites, compared to that of untreated DNA. The relative hybridization intensities of six probe-sets are shown for a representative fragment (IV). The ratio between the hybridization intensities of untreated and MSRE-treated sites was then used to generate quantitative methylation signals (V), which may transformed into methylation levels (%) based on experimental calibration curves. **b.** Target sequences of the MSREs included in the restriction endonucleases cocktail. **c.** Distribution of methylation levels at 1,461,753 informative genomic fragments probed on the Affymetrix 6.0 mapping microarray and of 1,341 genomic fragments of known unmethylated sites (1). **d.** Distribution of methylation levels (as in panel C) of case and control pools (average of 2 case and 2 control pools are shown). **e.** Distribution of methylation levels of young and old control pools. Data obtained using the Affymetrix SNP 6 (panels c and d) or the 250K (panel e) mapping array. **f-i:** the distribution of CpG sites among different fractions of the genome (color code), shown for all genomic CpGs (f), for the CpGs assessed by the microarray-based methylation assay (g), for the microarray-probed CpGs in case-control differentially methylated regions (h), and for the microarray-probed CpGs in T2DM-associated genomic blocks (i). The data show that the distribution of the microarray-probed CpGs is similar to the distribution of CpGs in the genome. Specifically, there is no bias towards the genomic fractions typical to the T2DM-associated blocks (i.e., gene-bodies) (i). Thus, the observed enrichment of DMRs in the T2D-associated blocks cannot be explained by an intrinsic bias of the differential methylation assay towards overrepresentation of these blocks.

Table S1. Methylation levels and case-control differences (absolute differences) of 93 CpGs embedded in T2DM-associated LD blocks. Total sequence reads are shown. Shaded lines indicate the methylation sites showing significant case-control differences (p<0.05, q<0.05).

Chr.	Position	Nearest gene	CTRL met. (%)	T2DM met. (%)	CTRL reads	T2DM reads	Met. Diff.	p-value	q-value
1	120312651	NOTCH2	89.40	91.57	491	463	2.17	0.2540	0.2904
1	120312791	NOTCH2	96.07	93.61	510	470	2.46	0.0805	0.1547
1	120328894	NOTCH2	98.60	98.26	2581	3043	0.35	0.2979	0.3147
1	120328907	NOTCH2	97.75	97.96	2586	3038	0.20	0.5981	0.4421
1	120328950	NOTCH2	73.79	74.54	2587	3048	0.75	0.5198	0.4315
1	120329026	NOTCH2	98.61	98.45	2582	3040	0.16	0.6278	0.4512
2	43590739	THADA	87.64	88.43	3972	4000	0.79	0.2780	0.3108
2	43590864	THADA	95.64	94.41	3947	3957	1.23	0.0120	0.0464
2	43591027	THADA	95.05	94.87	3962	3979	0.18	0.7129	0.4590
2	43591331	THADA	99.04	98.56	2615	2434	0.48	0.1128	0.1773
2	43591384	THADA	98.68	99.13	2566	2405	0.45	0.1304	0.1929
3	64678670	ADAMTS9	87.22	87.58	3585	3809	0.36	0.6402	0.4537
3	64678690	ADAMTS9	93.50	94.45	3585	3803	0.95	0.0861	0.1547
3	64678726	ADAMTS9	92.09	90.65	3590	3809	1.44	0.0282	0.0831
3	64678796	ADAMTS9	24.81	25.23	3587	3804	0.42	0.6734	0.4590
3	64681160	ADAMTS9	96.03	95.87	2900	3054	0.16	0.7549	0.4685
3	64681206	ADAMTS9	92.44	93.09	2900	3055	0.65	0.3339	0.3360
3	64681258	ADAMTS9	90.68	88.96	2865	2990	1.72	0.0297	0.0831
3	64685117	ADAMTS9	79.79	79.78	2267	2701	0.01	0.9946	0.5397
3	64685407	ADAMTS9	63.97	63.92	2232	2636	0.05	0.9699	0.5362
3	64686230	ADAMTS9	92.07	90.92	3924	4118	1.16	0.0629	0.1361
3	64686345	ADAMTS9	59.19	60.45	3926	4111	1.25	0.2519	0.2904
3	64687957	ADAMTS9	79.73	79.37	2393	2885	0.36	0.7501	0.4685
3	64688103	ADAMTS9	97.19	96.15	2388	2886	1.04	0.0374	0.0942
7	28141064	JAZF1	98.69	98.83	3745	3682	0.14	0.5784	0.4343
7	28141095	JAZF1	93.07	93.67	3741	3683	0.60	0.3003	0.3147
7	28143326	JAZF1	94.19	93.70	4302	3556	0.49	0.3647	0.3505
7	28143410	JAZF1	92.82	92.91	4307	3557	0.09	0.8726	0.5135
7	28143444	JAZF1	98.40	97.75	4304	3559	0.65	0.0357	0.0942
7	28143482	JAZF1	94.54	92.94	4305	3555	1.60	0.0034	0.0188
7	28143582	JAZF1	96.97	96.61	4257	3539	0.36	0.3692	0.3505
7	28143733	JAZF1	98.52	98.68	3919	4684	0.16	0.5332	0.4327
7	28143765	JAZF1	98.13	98.53	3916	4698	0.40	0.1518	0.2009
7	28143827	JAZF1	95.89	95.70	3919	4676	0.19	0.6554	0.4580
7	28143893	JAZF1	96.12	96.14	3919	4692	0.02	0.9537	0.5362
7	28143956	JAZF1	96.96	96.31	3918	4698	0.65	0.0986	0.1653
8	118247781	SLC30A8	85.12	84.83	4188	4040	0.30	0.7062	0.4590
8	118247902	SLC30A8	62.38	60.25	4136	4010	2.13	0.0483	0.1157
8	118257326	SLC30A8	85.61	83.43	5159	4888	2.18	0.0025	0.0188
8	118257358	SLC30A8	96.12	95.07	5130	4845	1.05	0.0102	0.0428
8	118257529	SLC30A8	76.73	77.79	2544	2576	1.07	0.3627	0.3505
8	118257972	SLC30A8	89.30	87.42	2496	3346	1.88	0.0274	0.0831
8	118257978	SLC30A8	91.86	91.97	2494	3326	0.11	0.8777	0.5135
8	118258012	SLC30A8	88.91	88.72	2497	3343	0.19	0.8215	0.4980
8	118258048	SLC30A8	78.44	79.27	2496	3343	0.83	0.4442	0.3921
8	118258573	SLC30A8	61.78	65.43	3370	2930	3.65	0.0027	0.0188

Table S1. ..end.

Chr.	Position	Nearest gene	CTRL met. (%)	T2DM met. (%)	CTRL reads	T2DM reads	Met. Diff.	p-value	q-value
10	94452858	HHEX	34.27	33.86	3011	1946	0.41	0.7635	0.4685
10	94452884	HHEX	76.38	75.62	3002	1907	0.76	0.5426	0.4334
10	114734658	TCF7L2	88.76	86.41	4618	3755	2.34	0.0012	0.0116
10	114734680	TCF7L2	97.21	96.68	4699	3829	0.53	0.1560	0.2012
10	114734701	TCF7L2	93.38	93.69	4700	3832	0.30	0.5718	0.4343
10	114734730	TCF7L2	96.82	96.22	4681	3816	0.60	0.1349	0.1939
10	114734749	TCF7L2	96.85	96.37	4667	3797	0.48	0.2201	0.2701
10	114734797	TCF7L2	91.90	92.62	4653	3807	0.72	0.2186	0.2701
10	114734825	TCF7L2	98.46	98.36	4690	3832	0.11	0.6975	0.4590
10	114738733	TCF7L2	96.47	96.23	3714	4885	0.24	0.5627	0.4343
10	114738765	TCF7L2	89.30	88.70	3719	4883	0.60	0.3801	0.3541
10	114738784	TCF7L2	97.63	98.13	3720	4883	0.50	0.1053	0.1710
10	114738858	TCF7L2	88.97	88.56	3718	4879	0.41	0.5541	0.4343
10	114738859	TCF7L2	86.13	86.19	2782	3969	0.06	0.9406	0.5362
10	114739026	TCF7L2	51.10	51.56	2775	3966	0.46	0.7077	0.4590
10	114739045	TCF7L2	50.84	52.97	2769	3957	2.13	0.0856	0.1547
10	114739311	TCF7L2	98.55	98.19	4633	1762	0.36	0.2912	0.3147
10	114739401	TCF7L2	99.25	98.53	4642	1768	0.72	0.0079	0.0361
10	114739403	TCF7L2	98.51	98.47	4645	1766	0.04	0.8977	0.5191
10	114743533	TCF7L2	95.47	95.76	3554	4788	0.29	0.5205	0.4315
10	114743543	TCF7L2	99.04	98.80	3536	4774	0.23	0.3184	0.3270
10	114743576	TCF7L2	97.74	97.74	3545	4783	0.00	0.9977	0.5397
10	114743580	TCF7L2	95.94	96.65	3545	4781	0.72	0.0846	0.1547
10	114743601	TCF7L2	94.52	92.59	3542	4777	1.93	0.0004	0.0055
10	114743664	TCF7L2	98.23	96.91	3551	4788	1.32	0.0001	0.0025
10	114743672	TCF7L2	98.68	98.16	3549	4787	0.52	0.0649	0.1361
10	114743783	TCF7L2	96.50	96.20	3314	3054	0.30	0.5231	0.4315
11	2805556	KCNQ1	36.49	34.51	3582	4795	1.98	0.0613	0.1361
11	2805588	KCNQ1	13.05	12.79	3585	4807	0.26	0.7207	0.4590
11	2805636	KCNQ1	20.12	18.19	3583	4792	1.92	0.0264	0.0831
11	2805655	KCNQ1	24.07	24.26	3580	4794	0.18	0.8468	0.5072
11	2805669	KCNQ1	14.86	14.46	3592	4806	0.40	0.6063	0.4421
11	2805692	KCNQ1	15.32	15.63	3584	4797	0.32	0.6910	0.4590
11	2805738	KCNQ1	23.21	24.60	3576	4797	1.39	0.1402	0.1959
11	2805785	KCNQ1	70.80	72.23	3590	4808	1.43	0.1507	0.2009
11	2805916	KCNQ1	90.23	92.20	2979	4180	1.97	0.0033	0.0188
11	2805938	KCNQ1	75.01	74.27	2981	4190	0.74	0.4808	0.4171
11	2806049	KCNQ1	88.06	90.97	2981	4187	2.92	0.0001	0.0015
11	2806079	KCNQ1	93.43	95.03	2968	4168	1.60	0.0038	0.0192
11	2806117	KCNQ1	60.26	61.16	2977	4184	0.90	0.4418	0.3921
11	17362226	KCNJ11	1.36	1.12	9547	9751	0.24	0.1286	0.1929
12	69942890	TSPAN8	89.92	88.86	4375	4774	1.07	0.0986	0.1653
12	69943012	TSPAN8	85.53	83.74	4367	4761	1.79	0.0182	0.0654
12	69943050	TSPAN8	89.41	89.44	4373	4762	0.03	0.9647	0.5362
12	69952996	TSPAN8/LGR5	80.78	77.88	328	2934	2.90	0.2273	0.2723
16	52366732	FTO	30.15	27.53	12180	11013	2.61	1E-05	0.0006
16	52366887	FTO	96.61	96.88	5844	5522	0.27	0.4163	0.3808

Table S2. Case-control methylation differences at clusters of neighboring CpG sites. Size and positions (hg18) of sequenced fragments, the number of CpG sites in each fragment, nearest gene name, total sequence reads, and case-control difference in the fraction of reads showing complete methylation (all CpG are fully methylated) out of all reads are shown. Shaded lines indicate the clusters showing significant case-control differences($p<0.05$, $q<0.05$).

Chr.	Start	End	CpGs (n)	Nearest gene	CTRL reads	T2DM reads	Full met. CTRL (%)	Full met. T2DM(%)	Diff.	p-value	q-value
1	120312604	120312792	2	NOTCH2	513	471	86.35	85.56	0.79	0.7207	0.6322
1	120328875	120329027	4	NOTCH2	2589	3049	70.30	71.30	1.00	0.4094	0.4621
2	43591303	43591439	2	THADA	2633	2443	97.76	97.70	0.05	0.9016	0.6322
3	64678648	64678822	4	ADAMTS9	3592	3813	20.10	20.25	0.14	0.8773	0.6322
3	64681139	64681259	3	ADAMTS9	2902	3058	81.19	80.64	0.54	0.5943	0.5919
3	64685113	64685434	2	ADAMTS9	2269	2706	53.99	53.58	0.41	0.7748	0.6322
3	64687953	64688132	2	ADAMTS9	2396	2887	77.71	76.34	1.37	0.2390	0.3678
7	28141049	28141097	2	JAZF1	3748	3688	91.95	92.60	0.65	0.2940	0.3829
7	28143298	28143593	5	JAZF1	4308	3561	82.84	82.76	0.09	0.9176	0.6322
8	118247780	118247919	2	SLC30A8	4205	4048	56.81	53.88	2.93	0.0074	0.0250
8	118257203	118257365	2	SLC30A8	5168	4895	83.39	80.75	2.64	0.0006	0.0047
8	118257477	118257689	4	SLC30A8	2500	3350	63.96	63.40	0.55	0.6628	0.6234
10	118257950	118258121	2	HHEX	3054	1964	31.43	28.92	2.51	0.0592	0.1113
10	118258486	118258613	7	TCF7L2	4704	3835	70.35	69.62	0.72	0.4672	0.4944
10	94452758	94452957	4	TCF7L2	3721	4888	76.43	76.51	0.08	0.9273	0.6322
10	114734572	114734828	3	TCF7L2	4651	1769	96.49	95.25	1.24	0.0207	0.0583
10	114738727	114738864	3	TCF7L2	2782	3971	33.03	34.30	1.27	0.2779	0.3829
10	114743495	114743680	7	TCF7L2	3558	4795	83.62	81.04	2.57	0.0024	0.0115
11	114739303	114739430	5	KCNQ1	2984	4193	36.93	39.18	2.25	0.0528	0.1113
11	114743495	114743680	8	KCNQ1	3596	4819	1.70	1.72	0.02	0.9336	0.6322
12	2805534	2805789	3	TSPAN8	4384	4780	71.03	69.12	1.91	0.0464	0.1113
16	52366689	52366689	2	FTO	12180	11013	29.84	27.28	2.55	1.71E-05	0.0003

Table S3. Methylation levels of the control individuals presented in figure 4.

#	Meth. (%)	Gender	Age	BMI
1	35.75	Male	40	32.72
2	30.14	Male	41	16.51
3	22.39	Male	41	31.62
4	33.44	Male	43	33.46
5	40.09	Female	43	22.68
6	32.52	Male	44	35.43
7	28.81	Female	44	30.84
8	30.59	Male	45	28.31
9	47.07	Female	45	21.17
10	18.42	Female	45	32.39
11	31.52	Male	46	32.05
12	46.41	Female	46	34.29
13	44.67	Female	47	23.31
14	39.14	Female	47	33.27
15	45.17	Male	48	22.49
16	25.72	Male	48	28.25
17	36.61	Male	49	28.06
18	33.28	Male	49	NA
19	35.22	Female	49	20.38
20	28.69	Male	50	29.05
21	40.83	Male	50	29.71
22	37.02	Female	50	30.48
23	37.33	Female	50	32.22
24	33.42	Male	51	26.51
25	33.31	Female	51	26.04
26	34.95	Female	51	22.01
27	38.5	Female	51	33.82
28	25.97	Male	52	NA
29	31.28	Female	52	35.28
30	24.63	Female	52	33.77
31	29.18	Male	53	26.57
32	40.81	Male	53	29.71
33	28.05	Female	53	37.75
34	36.15	Female	54	22.04
35	19.62	Female	54	32.04
36	44.09	Female	54	23.39
37	25.47	Female	54	32.86
38	23.69	Female	54	33.66
39	32.95	Female	54	37.85
40	31.11	Female	55	21.48
41	17.08	Female	55	24.36
42	10.01	Female	55	23.31
43	34.62	Female	55	22.98
44	34.79	Female	55	24.82
45	32.78	Female	55	38.03
46	27.09	Female	55	29.73
47	34.34	Male	56	NA
48	29.05	Male	56	NA
49	38.38	Male	56	NA
50	43.83	Female	56	41.62
51	33.25	Female	56	28.00
52	36.06	Female	56	34.12
53	35.99	Female	56	34.72
54	39.41	Female	56	30.30
55	29.93	Female	56	34.84
56	28.98	Female	56	25.17
57	28.19	Female	56	32.08
58	39.03	Female	56	42.81
59	40.5	Female	56	36.73
60	9.48	Female	56	31.14
61	25.98	Male	57	NA
62	22.09	Male	57	NA
63	32.56	Male	57	NA
64	39.63	Female	57	21.26
65	32.38	Female	57	56.92
66	29.51	Female	57	31.04
67	52.04	Female	57	44.44
68	22.7	Female	57	37.83
69	36.68	Female	57	29.38
70	8.9	Male	57	23.53
71	27.85	Male	58	NA
72	28.83	Male	58	NA
73	23.64	Male	58	NA
74	33.09	Male	58	23.94
75	43.55	Female	58	42.44
76	32.27	Female	58	29.27
77	35.56	Female	58	28.94
78	20.92	Female	58	48.58
79	33.88	Female	58	28.55
80	41.67	Female	58	24.88
81	38.64	Female	58	30.20
82	24.44	Male	59	23.80
83	18.71	Male	59	NA
84	33.71	Male	59	NA
85	40.74	Male	59	NA
86	31.15	Female	59	21.69
87	39.91	Female	59	27.25
88	29.53	Female	59	27.40
89	39.24	Female	59	36.63
90	35.73	Female	59	34.63
91	25.25	Female	59	31.89
92	34.99	Female	59	31.20
93	15.89	Female	59	24.80
94	28.81	Female	59	23.58
95	12.71	Female	59	39.92
96	33.07	Female	59	23.70
97	34.09	Female	59	32.76
98	31.39	Male	59	23.53
99	26.82	Female	59	23.44
100	24.65	Female	59	28.04
101	36.92	Female	60	31.64
102	33.26	Male	60	NA
103	29.09	Male	60	27.08
104	47.41	Female	60	25.40
105	38.93	Female	60	35.72
106	45.46	Female	60	30.57
107	47.25	Female	60	28.54
108	32.25	Female	60	32.80
109	38.83	Female	60	32.30
110	29.32	Female	60	29.63
111	35.83	Male	61	NA
112	20.55	Male	61	NA
113	42.24	Male	61	NA
114	25.68	Male	61	NA
115	25.74	Male	61	NA
116	36	Male	61	NA
117	43.85	Male	61	NA
118	40.84	Male	61	NA
119	24.91	Male	61	NA
120	37.27	Male	61	NA
121	34.55	Male	61	NA
122	29.85	Male	61	20.57
123	27.32	Male	61	25.16
124	30.69	Male	61	28.40
125	32.79	Female	61	29.44
126	36.75	Female	61	31.83
127	39.57	Female	61	33.98
128	32.63	Female	61	44.92
129	35.71	Female	61	29.00
130	31.42	Male	61	25.18
131	31.39	Male	62	NA
132	28.85	Male	62	NA
133	27.98	Male	62	NA
134	37.62	Male	62	NA
135	27.8	Male	62	NA
136	42.07	Male	62	NA
137	29.64	Male	62	NA
138	27.08	Male	62	28.33
139	48.81	Female	62	25.65
140	42.53	Female	62	22.41
141	22.14	Female	62	25.09
142	37.05	Female	62	21.35
143	48.8	Female	62	36.61
144	40.16	Female	62	35.49
145	23.08	Female	62	28.46
146	41.25	Female	62	33.98
147	29.26	Female	62	41.34
148	41.72	Female	62	35.61
149	34.12	Female	62	34.19
150	18.88	Male	62	24.80
151	40.1	Male	62	30.85
152	26.01	Male	63	NA
153	31.54	Male	63	NA
154	40.84	Male	63	NA
155	30.07	Male	63	20.99
156	41.9	Female	63	19.84
157	34.81	Female	63	25.32
158	40.67	Female	63	28.57
159	35.37	Female	63	24.70
160	34.88	Female	63	24.36
161	38.7	Female	63	35.52
162	18.94	Female	63	32.99
163	28.28	Female	63	35.28
164	27.59	Male	64	NA
165	25.54	Male	64	NA
166	30.69	Male	64	NA
167	44.61	Male	64	NA
168	26.87	Male	64	NA
169	32.87	Female	64	25.43
170	29.05	Female	64	32.66
171	32.04	Female	64	55.84
172	41.84	Female	64	27.32
173	41.33	Female	64	43.69
174	24.01	Female	64	38.44
175	13.77	Female	64	23.44
176	21.88	Male	65	27.51
177	23.37	Male	65	NA
178	43.07	Male	65	NA
179	23.68	Male	65	NA
180	27.83	Male	65	NA
181	29	Male	65	NA
182	34.82	Male	65	24.80
183	34.91	Male	65	24.77
184	39.84	Female	65	27.91
185	33.36	Female	65	33.04
186	24.48	Female	65	30.64
187	28.31	Female	65	28.65
188	27.49	Male	65	37.22
189	13.38	Male	66	NA
190	30.42	Male	66	NA
191	27.33	Male	66	NA
192	27.83	Male	66	NA
193	34.25	Male	66	NA
194	37.74	Female	66	27.91
195	35.47	Female	66	36.64
196	46.41	Female	66	21.82
197	47.22	Female	66	36.56
198	25.18	Female	66	29.30
199	22.71	Female	66	28.04
200	25.86	Male	66	24.17
201	33.16	Male	66	28.20
202	25.29	Male	67	NA
203	32.87	Male	67	NA
204	23.66	Male	67	NA
205	27.26	Male	67	NA
206	25.14	Male	67	NA
207	30.53	Male	67	21.10
208	40.01	Male	67	28.28
209	29.97	Male	67	32.66
210	38.25	Female	67	27.80
211	28.67	Female	67	22.83
212	19.83	Female	67	22.42
213	30.64	Female	67	27.72
214	23.34	Male	68	NA
215	27.37	Male	68	NA
216	14.37	Male	68	NA
217	24.5	Male	68	NA
218	26.09	Male	68	NA
219	33.82	Female	68	29.78
220	41.53	Female	68	23.01
221	24.98	Male	68	25.16
222	26.22	Male	68	27.77
223	26.8	Female	68	28.67
224	26.02	Female	68	34.38
225	26.23	Male	69	NA
226	20.58	Male	69	NA
227	22.17	Male	69	NA
228	29.31	Male	69	NA
229	37.08	Male	69	NA
230	27.6	Female	69	27.28
231	44.85	Female	69	27.20
232	37.07	Female	70	29.16
233	41.69	Female	70	32.65

Table S4. Methylation levels of the T2DM patients presented in figure 4.

#	Meth. (%)	Gender	Age	BMI
1	37.48	Female	43	23.19
2	19.96	Female	43	26.22
3	25.07	Male	45	31.96
4	20.83	Male	45	28.36
5	36.95	Male	45	28.81
6	25.53	Male	46	20.88
7	47.79	Male	46	37.04
8	27.79	Male	46	28.73
9	26.83	Female	46	36.00
10	24.16	Female	46	32.99
11	24.87	Female	47	25.01
12	27.47	Male	48	26.99
13	24.1	Male	49	32.41
14	20.36	Male	49	20.98
15	25.05	Male	49	28.37
16	32.66	Female	49	37.10
17	20.24	Male	50	24.73
18	28.35	Male	50	28.31
19	31.1	Male	50	32.77
20	21.57	Female	50	33.59
21	27.29	Male	51	28.60
22	25.81	Male	51	27.77
23	31.04	Male	52	32.45
24	35.27	Female	52	36.00
25	30.79	Female	52	24.52
26	26.99	Male	53	32.24
27	18.97	Male	53	32.37
28	20.12	Male	53	27.72
29	30.6	Male	53	33.31
30	31.95	Female	53	37.28
31	28.64	Female	53	24.09
32	22.68	Female	53	36.74
33	33.68	Female	53	28.74
34	22.18	Male	54	32.28
35	34.54	Male	54	35.43
36	39.88	Female	54	28.04
37	28.05	Female	54	32.86
38	42.86	Female	54	28.19
39	31.82	Female	54	36.13
40	28.71	Female	54	36.72
41	20.54	Female	54	28.73
42	34.89	Female	54	23.38
43	27.91	Male	55	35.59
44	35.42	Male	55	21.30
45	23.52	Male	55	24.82
46	27.73	Male	55	28.41
47	22.7	Female	55	33.46
48	48.77	Female	55	20.89
49	19.61	Female	55	23.80
50	29.55	Female	55	37.47
51	38.6	Female	55	32.44
52	32.22	Female	55	32.69
53	29.35	Male	56	32.28
54	16.55	Male	56	25.73
55	31.97	Female	56	32.65
56	40.93	Female	56	28.28
57	29.57	Female	56	28.52
58	26.58	Male	57	23.60
59	34.3	Male	57	25.50
60	23.74	Male	57	32.79
61	24.21	Male	57	28.68
62	33.45	Male	57	28.08
63	22.63	Male	57	32.95
64	19.21	Male	57	24.62
65	23.98	Male	57	27.47
66	30.1	Male	57	30.32
67	45.12	Female	57	22.31
68	40.08	Female	57	28.91
69	18.88	Female	57	24.90
70	44.44	Female	57	22.66
71	24.28	Male	58	28.73
72	24.03	Male	58	25.26
73	35.83	Male	58	23.41
74	32.91	Female	58	32.47
75	26.47	Female	58	28.70
76	38.74	Female	58	33.06
77	38.82	Female	58	31.25
78	17.64	Female	58	44.92
79	19.69	Female	58	39.45
80	26.91	Male	59	23.66
81	29.6	Male	59	28.41
82	26	Male	59	37.32
83	25.97	Male	59	28.62
84	33.36	Female	59	24.34
85	42.31	Female	59	37.44
86	35.24	Female	59	36.92
87	28.98	Female	59	32.43
88	34.98	Female	59	28.34
89	32.17	Male	60	28.72
90	27.07	Male	60	24.91
91	25.83	Male	60	22.34
92	16.22	Male	60	27.64
93	24.19	Female	60	33.20
94	28.02	Female	60	33.59
95	33.13	Female	60	31.25
96	15.53	Male	61	23.62
97	23.17	Male	61	24.82
98	24.79	Female	61	21.76
99	28.85	Female	61	33.39
100	21.62	Female	61	34.48
101	21.5	Female	61	30.80
102	27.68	Male	62	24.45
103	36.68	Male	62	21.80
104	20.09	Male	62	22.34
105	39.06	Female	62	37.46
106	30.18	Female	62	21.64
107	33.3	Female	62	28.30
108	31.44	Female	62	37.72
109	38.19	Female	62	32.42
110	43.88	Female	62	30.08
111	27.99	Male	63	19.15
112	22.29	Male	63	24.34
113	23.56	Male	63	32.65
114	29.95	Male	63	28.06
115	24.98	Male	63	37.52
116	25.58	Male	63	24.52
117	28.11	Male	63	30.82
118	36.69	Female	63	33.59
119	36.67	Female	63	22.43
120	23.95	Female	63	28.33
121	32.1	Female	63	28.70
122	22.35	Male	64	24.24
123	28.63	Male	64	35.38
124	30.34	Male	64	36.44
125	23.22	Male	64	32.32
126	26.89	Male	64	22.84
127	32.14	Male	64	27.78
128	18.02	Female	64	21.64
129	29.61	Female	64	22.66
130	29.96	Female	64	24.35
131	27.14	Female	64	28.48
132	28.47	Female	64	25.78
133	28.07	Female	64	30.41
134	28.79	Male	65	22.59
135	28.51	Male	65	32.39
136	25.08	Male	65	23.94
137	34.15	Male	65	28.08
138	33.46	Male	65	37.56
139	23.97	Male	65	28.37
140	29.22	Male	65	29.05
141	32.1	Female	65	24.31
142	26.6	Female	65	25.04
143	30.42	Female	65	32.69
144	36.36	Male	66	25.16
145	28.32	Male	66	28.09
146	31.86	Male	66	21.05
147	14.8	Male	66	22.98
148	29.28	Male	66	22.99
149	19.15	Male	66	23.88
150	28.7	Male	66	32.08
151	21.78	Male	66	29.04
152	22.54	Male	66	33.73
153	35.83	Female	66	28.65
154	34.62	Female	66	20.94
155	33.12	Female	66	35.55
156	17.45	Female	66	24.13
157	30.86	Female	66	22.77
158	22.85	Female	66	33.46
159	35.03	Female	66	28.44
160	20.88	Male	67	24.69
161	14.66	Male	67	25.85
162	28.88	Male	67	23.53
163	20.25	Female	67	37.29
164	21.13	Female	67	29.00
165	28.34	Female	67	32.35
166	28.72	Female	67	33.15
167	28.48	Female	67	32.47
168	20.04	Female	67	35.34
169	29.57	Female	67	33.43
170	27.74	Female	67	32.39
171	23.55	Male	68	22.41
172	12.51	Male	68	24.00
173	22.8	Male	68	31.95
174	21.73	Male	68	32.00
175	31.38	Male	68	31.35
176	25.68	Female	68	28.04
177	28.39	Female	68	28.13
178	27.46	Female	68	35.30
179	37.75	Female	68	20.76
180	30.62	Female	68	28.35
181	41.73	Female	68	36.89
182	49.3	Female	68	24.61
183	33.23	Female	68	25.91
184	16.37	Male	69	21.91
185	29.53	Male	69	33.02
186	29.07	Male	69	28.33
187	28.22	Male	69	23.88
188	18.92	Female	69	36.89
189	55.16	Female	69	28.13
190	27.77	Female	69	28.80
191	45.51	Female	69	28.37
192	37.73	Female	69	37.46
193	31.84	Female	69	25.97
194	22.8	Male	70	37.02
195	18.56	Male	70	24.39
196	16.79	Male	70	24.34
197	22.63	Male	70	22.50
198	38.99	Female	70	35.88

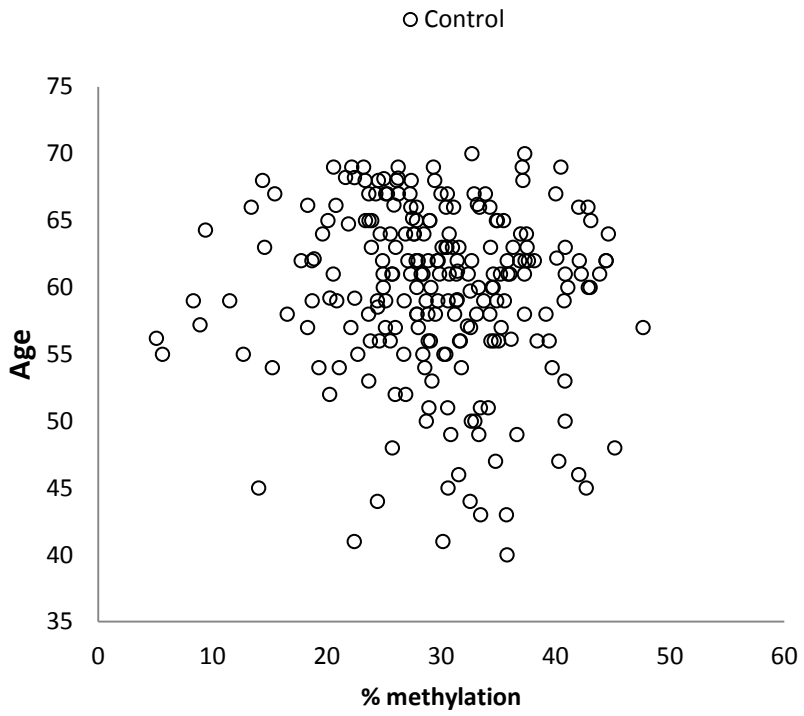


Figure S2. The ages of the 233 controls presented in figure 4, versus their methylation levels. No significant correlation ($R^2 = 0.011$) appeared between methylation and age within the examined age range.

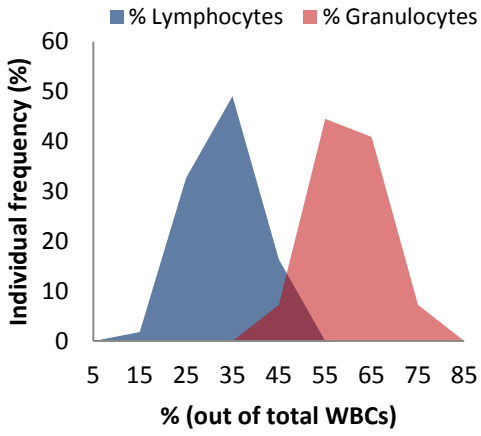
Table S5. Methylation levels, differential blood counts, and glucose levels of the control participants in the Jerusalem LRC longitudinal (cohort) study.

			Age 30						Age 43	
#	Meth. (%)	Gender	BMI	Lymph (%)	Gran (%)	Mono (%)	Fasting Glucose	2h Glucose	Fasting Glucose	2h Glucose
1	34.32	Female	33.83	30.6			98	81	81	96
2	39.52	Female	21.68	32.7	65.4	1.9	82	114	82	121
3	38.01	Female	20.92	33.9	60.5	5.6	82	88	82	85
4	26.26	Female	21.83	25.6	67.4	7.0	91	85	83	93
5	40.40	Female	20.03	30.3	NA	NA	84	74	84	77
6	49.92	Female	22.47	43.3	49.9	6.8	97	91	86	123
7	51.39	Male	22.56	46.9	45.5	7.6	88	78	86	75
8	21.69	Male	21.43	16.9	76.2	6.9	96	96	86	68
9	42.24	Female	22.58	40.7	54.5	4.8	94	90	86	66
10	44.28	Male	25.81	36.3	56.9	6.8	85	35	86	66
11	36.33	Female	23.85	38.2	54.1	7.7	84	108	87	132
12	39.83	Female	27.36	34.6	58.3	7.1	91	102	87	85
13	39.24	Male	24.40	41.0	55.9	3.1	86	76	88	108
14	25.00	Male	23.66	22.1	68.5	9.4	82	79	88	102
15	34.81	Male	23.95	24.7	70.3	5.0	87	86	89	119
16	39.37	Male	23.23	30.2	61.1	8.7	87	89	89	89
17	28.25	Male	23.89	24.1	73.1	2.8	94	61	89	80
18	43.29	Male	24.27	35.6	58.6	5.8	85	62	89	78
19	34.15	Male	20.70	26.1	65.7	8.2	88	60	90	122
20	33.74	Male	28.36	28.1	65.5	6.4	84	86	90	116
21	42.32	Male	24.81	35.9	57.4	6.7	90	93	90	90
22	40.61	Male	20.73	37.3	56.7	6.0	91	78	90	82
23	46.13	Male	21.43	39.8	54.1	6.1	94	56	91	133
24	37.78	Male	20.97	37.1	57.0	5.9	88	107	91	109
25	33.18	Male	21.18	37.9	54.9	7.2	80	76	91	89
26	35.98	Female	23.91	25.9	69.9	4.2	95	99	92	120
27	39.55	Male	21.55	40.7	50.0	9.3	96	81	92	102
28	36.10	Male	29.27	30.2	62.8	7.0	98	90	92	86
29	46.95	Male	20.04	49.2	43.8	7.0	89	87	92	76
30	39.76	Male	25.02	30.9	57.8	11.3	82	56	93	94
31	51.86	Female	20.06	40.8	57.3	1.9	84	86	93	77
32	35.44	Male	23.70	35.4	56.3	8.3	81	53	93	61
33	40.93	Male	22.68	34.8	58.0	7.2	88	73	94	102
34	46.74	Male	22.91	35.7	56.2	8.1	90	96	94	94
35	24.27	Female	22.45	25.4	NA	NA	89	77	95	127
36	30.52	Male	24.15	23.9	71.8	4.3	91	132	95	122
37	44.05	Male	25.52	47.1	50.2	2.7	99	108	95	113
38	44.34	Male	22.87	29.4	62.6	8.0	97	38	95	110
39	37.30	Female	21.90	34.6	53.1	12.3	90	82	95	101
40	34.67	Male	20.98	34.4	59.0	6.6	89	85	95	98
41	39.15	Male	22.53	35.9	59.3	4.8	93	89	95	89
42	33.52	Male	20.87	34.1	57.4	8.5	92	66	95	68
43	35.27	Male	31.58	33.4	60.7	5.9	91	91	95	63
44	37.23	Male	23.89	29.6	65.2	5.2	82	78	96	NA
45	42.52	Male	23.87	29.3	66.9	3.8	88	133	96	137
46	43.40	Male	19.71	39.5	NA	NA	96	117	96	118
47	40.03	Male	21.78	47.2	43.0	9.8	85	66	96	97
48	27.86	Male	22.62	30.7	60.2	9.1	95	94	96	92
49	30.37	Male	27.69	24.5	69.9	5.6	87	97	96	88
50	37.93	Male	23.21	41.0	48.9	10.1	93	84	96	79
51	23.95	Male	23.06	24.9	67.6	7.5	87	76	96	75
52	34.56	Male	27.34	30.7	67.6	1.7	84	70	97	136
53	43.03	Male	24.25	37.0	57.1	5.9	97	98	97	97
54	34.06	Male	29.98	32.1	60.9	7.0	80	57	98	111
55	34.35	Male	24.30	21.3	72.8	5.9	99	107	98	91
56	45.05	Male	24.14	38.5	52.4	9.1	97	76	98	91
57	43.25	Male	31.59	38.7	54.9	6.4	99	70	98	90
58	34.58	Male	24.05	24.8	70.7	4.5	95	98	98	87
59	48.86	Male	19.18	49.4	46.0	4.6	93	102	98	86
60	39.90	Male	27.42	25.8	67.0	7.2	97	67	98	64
61	26.25	Female	23.21	28.6	64.2	7.2	96	98	99	107
62	33.40	Male	23.19	29.7	65.1	5.2	95	97	99	106
63	44.53	Female	27.18	28.8	67.5	3.7	93	95	99	74
64	48.58	Female	25.09	37.3	55.7	7.0	81	79	99	57

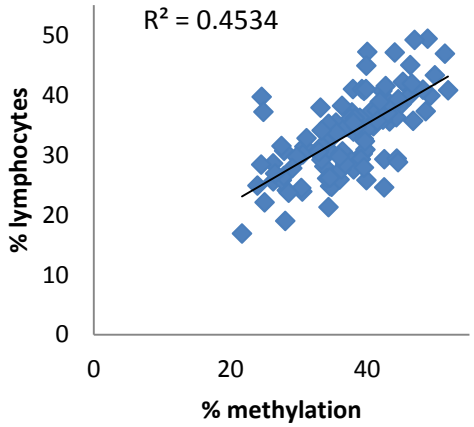
Table S6. Methylation levels, differential blood counts, and glucose levels of the incidence cases of IGM/T2DM participants in the Jerusalem LRC longitudinal (cohort) study.

			Age 30						Age 43	
#	Meth. (%)	Gender	BMI	Lymph (%)	Gran (%)	Mono (%)	Fasting Glucose	2h Glucose	Fasting Glucose	2h Glucose
1	46.56	Female	21.73	41.9	53.4	4.7	82	80	81	151
2	31.34	Female	22.19	22.9	NA	NA	48	82	90	152
3	29.70	Male	26.25	29.7	67.2	3.1	85	93	92	152
4	25.18	Male	33.54	26.3	NA	NA	98	126	93	152
5	30.58	Male	26.79	30.1	63.2	6.7	88	95	94	148
6	42.50	Male	29.07	24.6	69.8	5.6	95	89	94	143
7	41.45	Female	20.19	37.4	56.4	6.2	97	93	94	141
8	35.74	Male	21.09	31.3	NA	NA	94	80	95	187
9	36.40	Male	24.91	30.6	62.4	7.0	79	95	96	197
10	34.36	Female	22.30	35.2	59.4	5.4	82	69	96	143
11	28.52	Female	19.69	23.7	66.7	9.6	77	76	97	163
12	41.86	Male	30.69	38.9	54.5	6.6	85	87	97	157
13	33.39	Male	21.40	31.5	61.4	7.1	93	88	97	143
14	29.05	Male	26.50	27.8	67.2	5.0	87	112	98	169
15	39.54	Female	23.65	27.9	65.0	7.1	95	98	100	147
16	44.56	Male	18.33	39.3	52.1	8.6	91	88	101	157
17	33.35	Male	25.81	34.1	58.6	7.3	85	59	102	148
18	24.57	Male	25.50	39.7	52.3	8.0	97	66	104	151
19	35.64	Male	26.20	29.5	NA	NA	93	121	104	141
20	36.23	Male	28.93	27.6	64.4	8.0	86	120	104	148
21	41.51	Female	32.17	35.7	55.4	8.9	90	117	105	212
22	36.79	Male	20.60	28.5	66.1	5.4	90	74	106	151
23	27.53	Male	22.25	25.8	64.6	9.6	96	113	107	141
24	38.21	Male	31.43	36.1	56.9	7.0	93	113	107	248
25	48.17	Male	27.11	40.9	53.4	5.7	94	113	109	158
26	27.47	Female	25.82	31.5	65.3	3.2	84	81	110	74
27	28.02	Male	23.34	19.0	74.0	7.0	88	91	110	82
28	29.99	Female	23.58	29.6	67.7	2.7	89	109	110	103
29	38.48	Male	22.91	34.7	57.2	8.1	94	117	110	88
30	45.24	Male	26.74	42.2	51.8	6.0	97	93	110	173
31	32.91	Male	23.90	30.5	66.9	2.6	94	92	111	105
32	34.54	Male	20.26	26.2	70.6	3.2	98	72	111	92
33	33.25	Male	29.72	34.4	NA	NA	93	92	112	142
34	33.32	Male	30.13	29.2	64.1	NA	95	137	112	106
35	36.94	Female	25.82	34.7	59.7	5.6	84	76	112	139
36	37.55	Male	25.22	36.1	55.9	8.0	93	74	112	95
37	39.84	Male	32.17	41.0	50.9	8.1	94	106	112	147
38	40.04	Male	22.73	34.4	56.2	9.4	93	92	112	112
39	42.72	Male	25.55	41.5	55.4	3.1	99	81	112	101
40	24.50	Male	27.77	28.4	64.3	7.3	94	85	113	99
41	32.28	Male	25.61	30.7	NA	NA	84	98	113	102
42	38.94	Male	22.68	36.2	55.8	8.0	97	68	113	104
43	26.54	Female	22.39	26.9	66.6	6.5	95	124	114	**NA**
44	30.81	Female	19.41	31.4	62.0	6.6	89	88	114	142
45	38.00	Male	24.74	35.4	56.4	8.2	96	70	114	73
46	39.89	Male	25.23	44.9	44.7	10.4	98	90	114	60
47	31.14	Male	27.84	32.8	57.6	9.6	98	118	115	174
48	35.54	Male	28.63	33.7	63.1	3.2	98	82	115	115
49	44.81	Male	16.62	39.0	NA	NA	89	58	115	100
50	27.82	Male	25.28	26.5	66.7	6.8	99	91	117	113
51	35.96	Male	33.15	29.7	65.7	4.6	92	82	117	92
52	37.13	Male	27.65	41.5	NA	NA	93	120	119	119
53	39.69	Female	30.43	32.3	59.7	8.0	99	109	124	144
54	46.32	Male	34.61	45.0	50.1	4.9	97	111	125	201
55	24.83	Male	28.73	37.2	54.4	8.4	77	78	126	171
56	39.09	Male	32.31	29.3	62.9	7.8	95	108	132	294
57	49.28	Female	39.92	39.9	53.9	6.2	92	96	135	NA
58	38.03	Male	26.12	27.6	64.4	8.0	97	60	363	NA

a



b



c

	Avg. %				Predicted methylation (%)			Bound (for all)	
	All	Ctrl	Case	**SD (for all)**	All	Ctrl	Case	Lower	Upper
Lymphocytes	33.36	33.59	33.10	6.63	83.67	84.50	81.24	73.39	93.96
Granulocytes	60.11	59.96	60.29	7.06	15.21	16.34	15.06	9.68	20.96
Monocytes	6.53	6.46	6.62	2.12	0	0	0	0	43.45

d

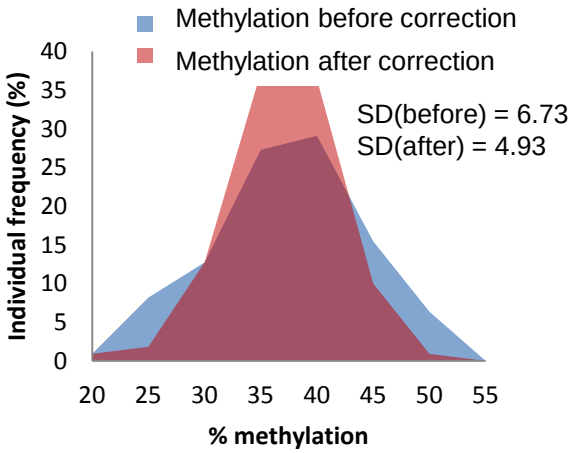


Figure S3. **Lineage-specific methylation levels in blood.** Analyses of 110 individuals from the Jerusalem Lipid Research Clinic (longitudinal) study for which we obtained full baseline blood counts and methylation levels. **a**. Distribution of lymphocyte and granulocyte percentages (out of total white blood cells) among the110 participates. **b.** Correlation between the percentage of lymphocytes and methylation levels. **c**. Prediction of lineage-specific methylation levels, based on the correlation presented in panel b (methods).**d.** Adjustment of methylation levels for blood counts decreases the between-individual variance by 26.7%.

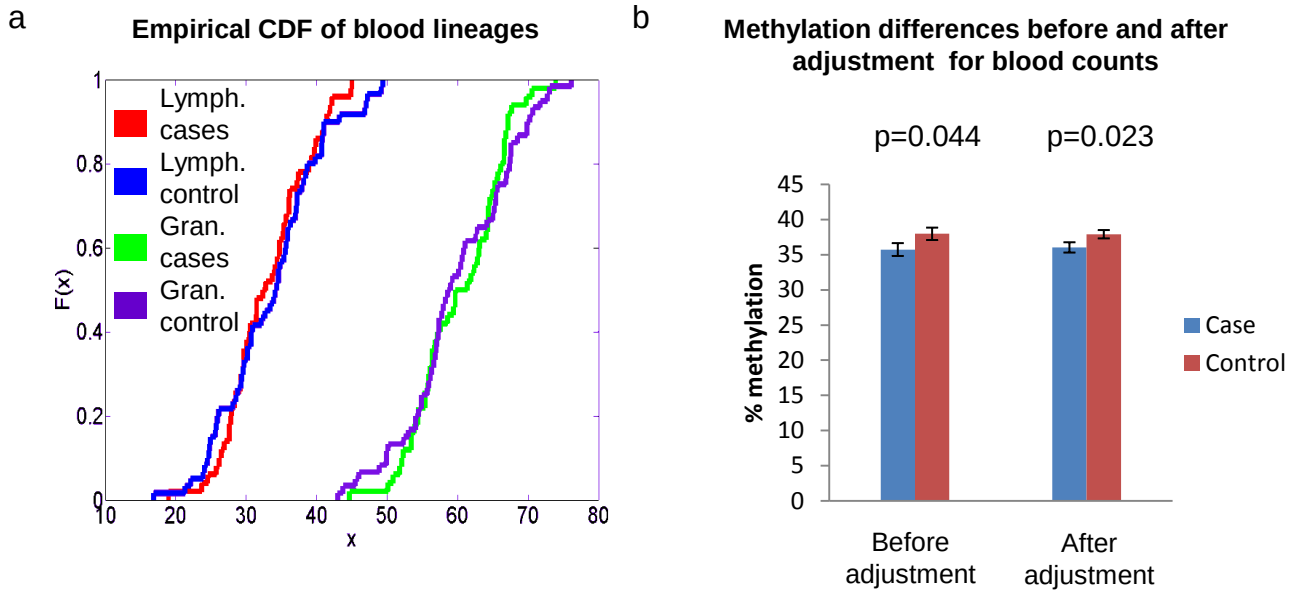


Figure S4. **Differential methylation between blood cell lineages did not confound the association of baseline methylation with incidence of IGM/T2DM.** Analyses of 110 individuals from the Jerusalem Lipid Research Clinic (longitudinal) study for which we obtained baseline blood counts of lymphocytes, granulocytes and monocytes and methylation levels. **a**. Empirical cumulative distribution functions (CDF) shows no significant differences (K-S tests>0.5) in blood counts between 50 cases (who later developed impaired glucose metabolism) and 60 healthy subjects. **b**. Methylation differences between cases and control before and after adjustment for differential blood counts. The data show that the case-control methylation difference is somewhat smaller following adjustment to blood counts, but, due to the reduced between-individual variance as presented in Figure S3d, cases and controls are even more significantly distinguished following the adjustment. Note that the adjusted p-value differs slightly from the one presented in Figure 6: Figure 6 refers to 122 individuals with lymphocyte counts, while the data presented here refer to the 110 individuals out of the 122 for which we also obtained granulocytes and monocytes counts.

Note S1. As many cell types exhibit lineage-specific methylation levels, control for a possible confounding effect of cell type composition in the examined tissue on case-control methylation differences should be undertaken (large databases consisting of DNA samples from a singular cell type are virtually unavailable). In the case of the *FTO* methylation site (Figure 3a) we indeed observed large methylation differences between the two dominant white blood cell (WBC) lineages, lymphocytes and granulocytes (Figure S3a-c). Differences in the counts of these two cell types between healthy subjects explained 26.7% of the inter-individual methylation variance in whole blood samples (figure S3d) (other blood lineages also showed specific methylation levels, but their contribution is very limited since they account for only a small fraction of the blood cell population). However, adjustment for this effect did not weaken the association of baseline methylation levels with the incidence of IGM/DM (Figure S4b).

We also searched the literature for reports on differences in blood counts between T2DM patients and normal individuals. While T2DM was associated with higher WBC counts relative to controls (2-3), significant differences in lymphocytes to granulocytes ratio were not reported. We have further contacted the author of a recent large meta-analysis on this issue (4) and confirmed that indeed no such differences were observed (5). Thus, differences in the differential blood counts between cases and non-cases, both in our cohort as well as in many other large cohorts, cannot explain the observed T2DM hypomethylation finding.

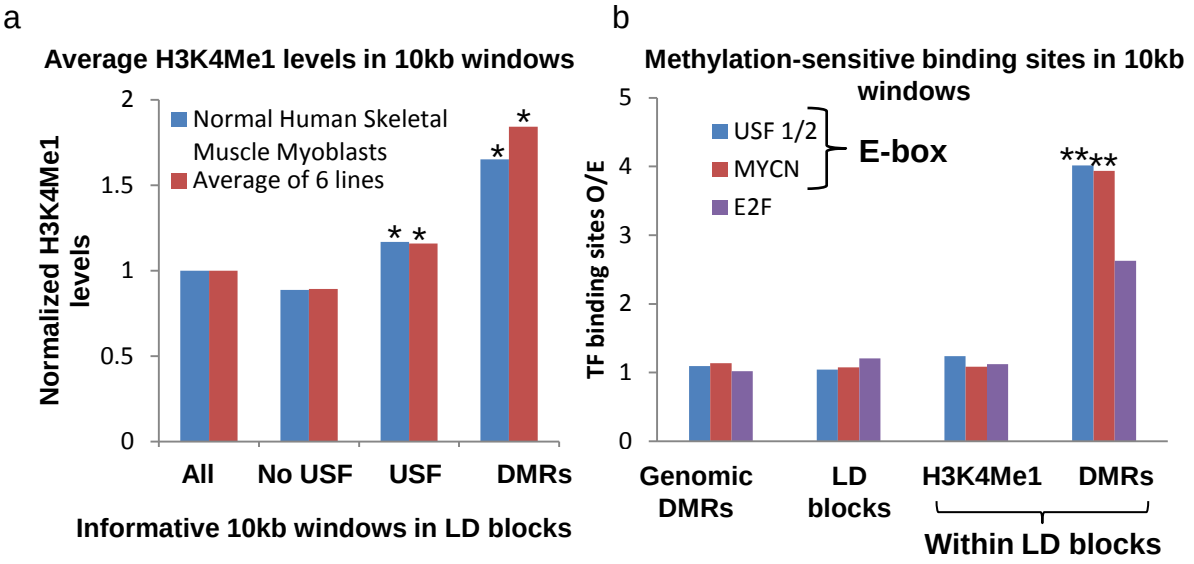


Figure S5. Same analyses as in figure 7 c-d, but for stringent DMRs rather for all DMRs. **a.** Average levels of H3K4Me1 marks in10kb windows along the T2DM LD blocks, in windows without or with USF1/2 binding sites, and in DMRs. Asterisks denote significant enrichment (P<0.05). **b.** The ratio of observed/expected numbers of binding sites (expectation is based on the frequency across the genome) in all genomic DMRs, in LD block windows, in LD block windows enriched with H3K4Me1 marks, and in LD block DMRs. Double asterisks denote significant enrichment (P<0.01).

The only major difference between the results of this analysis and the results of the analysis presented in figure 7 was that among stringent DMR, binding sites for the E2F transcription factor were not significantly enriched (p=0.099). This may be due to the smaller number of stringent DMRs compared to the number of all DMRs. USF1/2 were the only methylation-sensitive transcription factors that showed enrichment at p-values <0.001 in both regular and stringent DMRs.

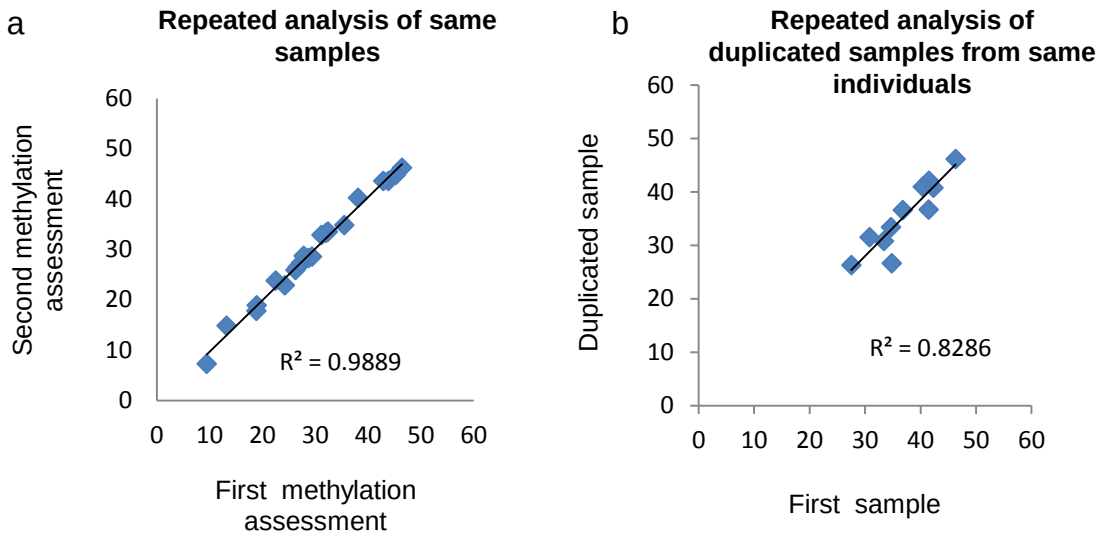


Figure S6. **Assay reproducibility and cohort quality. a.** Replicated pyrosequencing analyses of 21 samples. The average difference between replicates was 0.89% and the correlation between the sets was R^2=0.99. **b.** Pyrosequencing analyses of duplicated samples obtained from11 randomly-selected participants out of the 122 participants in the longitudinal study. All samples were treated together through all the analysis stages.

Supplementary references:

1. Straussman R. et al. (2009) Developmental programming of CpG island methylation profiles in the human genome. Nat Struct Mol Biol. **16**, 564-571.

2. Vozarova, B. et al. (2002) High white blood cell count is associated with a worsening of insulin sensitivity and predicts the development of type 2 diabetes. Diabetes **51**, 455-461.

3. Tsai, J. C. et al. (2007) Association of peripheral total and differential leukocyte counts with metabolic syndrome and risk of ischemic cardiovascular diseases in patients with type 2 diabetes mellitus. Diabetes Metab Res Rev **23**, 111-118.

4. Gkrania-Klotsas, E. et al. (2010) Differential white blood cell count and type 2 diabetes: systematic review and meta-analysis of cross-sectional and prospective studies. PLoS One **5**, e13405.

5. E. Gkrania-Klotsas, personal communication.