Supplemental Material to:

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Cross-tissue comparisons of leptin and adiponectin: DNA methylation profiles

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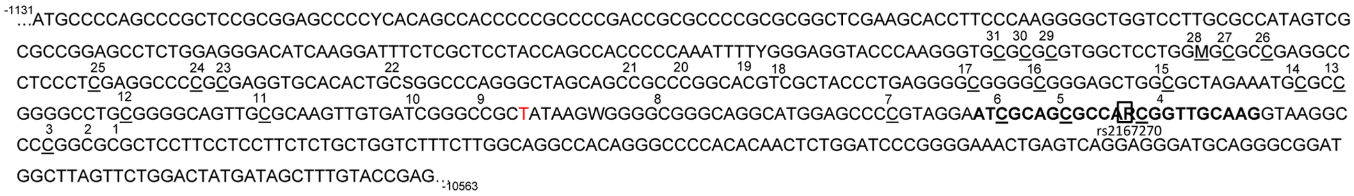
Table S1. Characteristics of subjects according to sex

	Men (*n* = 33)		**Women (*n* = 40)**	
	Mean ± SD	**Min–max**	**Mean ± SD**	**Min–max**
Age (y)	35.2 ± 6.9	21.5–49.7	34.2 ± 7.3	21.4–53.8
Height (m)	1.76 ± 0.08	1.59–1.90	1.62 ± 0.06**	1.50–1.76
Weight (kg)	158.7 ± 21.0	125.8–200.2	127.5 ± 17.8**	90.2–168.6
BMI (kg/m²)	51.1 ± 5.3	42.0–59.5	48.4 ± 6.3*	40.0–60.0
Hip circumference (cm)	148.5 ± 14.0	123.0–173.0	147.0 ± 14.1	126.0–193.0
Waist circumference (cm)	150.6 ± 13.4	129.0–180.0	130.0 ± 12.8**	99.0–152.0
Waist:hip ratio	1.02 ± 0.08	0.82-1.16	0.89 ± 0.09**	0.67–1.17
Fasting glucose levels (mmol/L)	5.69 ± 1.05	4.20–8.70	5.36 ± 1.04	4.00–8.70

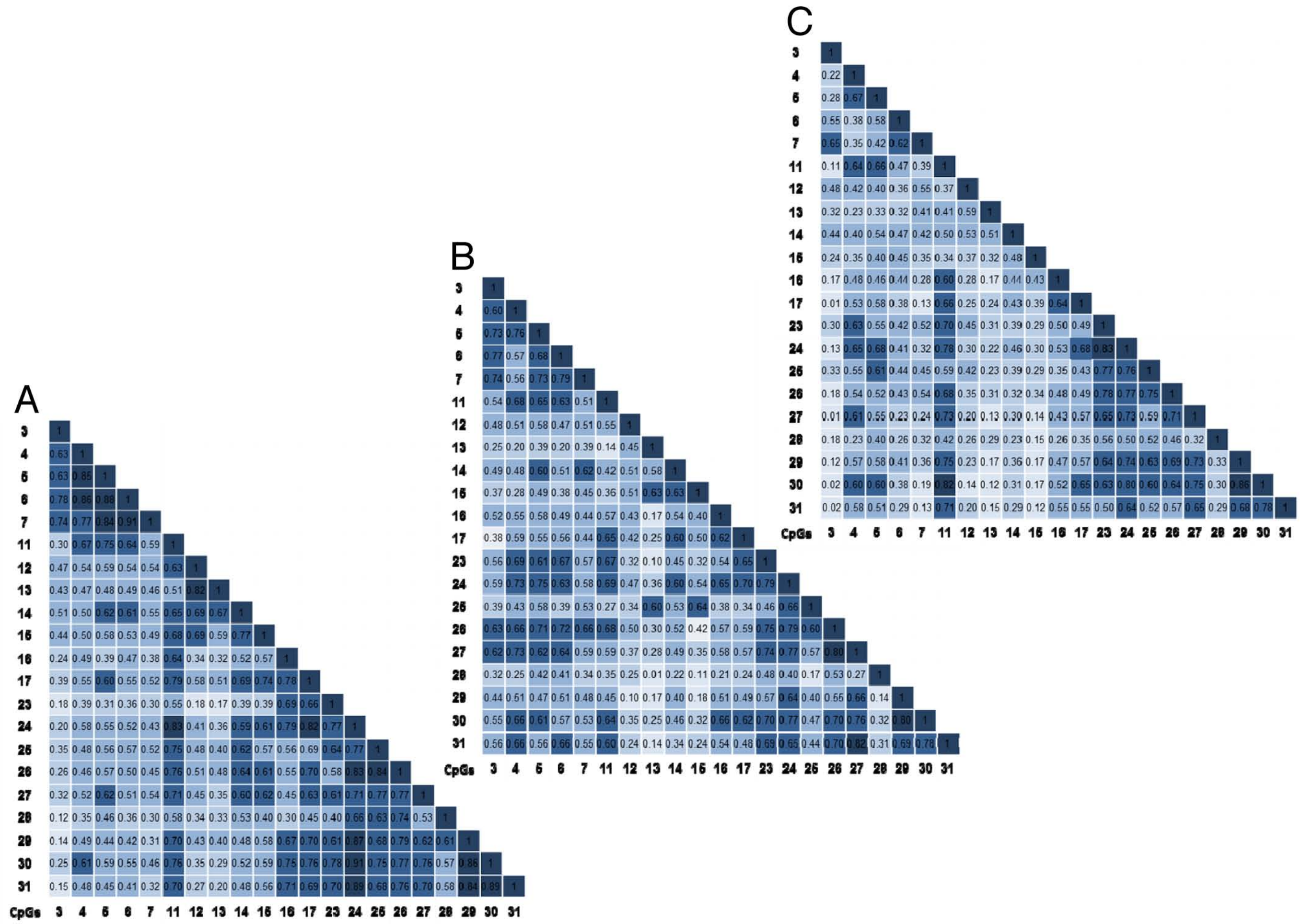
BMI, body mass index; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue. Values are significantly different between men and women: **$P < 0.01$; *$P < 0.05$.



-1131...ATGCCCCAGCCCGCTCCGCGGAGCCCCYCACAGCCACCCCCGCCCCGACCGCGCCCCGCGCGGCTCGAAGCACCTTCCCAAGGGGCTGGTCCTTGCGCCATAGTCG
CGCCGGAGCCTCTGGAGGGACATCAAGGATTTCTCGCTCCTACCAGCCACCCCCAAATTTTYGGGAGGTACCCAAGGGTGCGCGCGTGGCTCCTGGMGCGCCGAGGCC
CTCCCTCGAGGCCCCGCGAGGTGCACACTGCSGGCCCAGGGCTAGCAGCCGCCCGGCACGTCGCTACCCTGAGGGGCGGGGCGGGAGCTGGCGCTAGAAATGCGCC
GGGGCCTGCGGGGCAGTTGCGCAAGTTGTGATCGGGCCGCTATAAGWGGGGCGGGCAGGCATGGAGCCCCGTAGGA**ATCGCAGCGCCARCGGTTGCAAG**GTAAGGC
CCCGGCGCGCTCCTTCCTCCTTCTCTGCTGGTCTTTCTTGGCAGGCCACAGGGCCCCACACAACTCTGGATCCCGGGGAAACTGAGTCAGGAGGGATGCAGGGCGGAT
GGCTTAGTTCTGGACTATGATAGCTTTGTACCGAG...-10563

	Mean DNA methylation levels ± SD (%)			*P*-value (paired sample T-Test)		
	SAT	VAT	Blood	SAT vs VAT	SAT vs blood	VAT vs blood
CpG3	34.6 ± 5.2	35.8 ± 4.7	34.6 ± 6.9	6.75E-02	0.97	1.54E-01
CpG4	18.4 ± 3.5	18.8 ± 3.2	29.2 ± 7.8	3.28E-01	1.39E-17	1.46E-18
CpG5	17.5 ± 3.0	17.0 ± 2.8	24.4 ± 6.5	1.50E-01	1.60E-12	9.66E-15
CpG6	16.5 ± 3.6	16.5 ± 3.2	23.2 ± 6.5	9.79E-01	2.52E-11	7.38E-13
CpG7	16.4 ± 3.4	15.3 ± 2.5	21.0 ± 6.0	5.89E-03	3.00E-07	7.83E-12
CpG11	24.5 ± 2.8	23.9 ± 4.1	40.5 ± 8.5	1.40E-01	3.41E-28	9.86E-28
CpG12	8.6 ± 1.4	7.6 ± 1.2	10.0 ± 2.7	3.99E-06	3.06E-05	7.16E-11
CpG13	9.7 ± 1.7	6.8 ± 1.2	7.9 ± 2.5	2.14E-19	5.32E-07	4.05E-04
CpG14	8.6 ± 1.4	6.1 ± 1.1	8.8 ± 2.7	3.84E-20	0.61	3.50E-13
CpG15	7.7 ± 1.3	6.3 ± 0.9	9.0 ± 2.3	2.21E-11	2.08E-05	7.52E-16
CpG16	6.4 ± 1.3	5.7 ± 1.2	10.3 ± 3.7	1.21E-04	1.42E-15	1.59E-17
CpG17	10.7 ± 2.1	10.0 ± 2.1	17.7 ± 5.2	1.85E-02	4.73E-22	9.11E-21
CpG23	8.8 ± 1.8	9.5 ± 1.9	16.7 ± 4.7	2.41E-03	4.22E-24	1.30E-20
CpG24	16.8 ± 2.7	15.9 ± 3.1	28.6 ± 7.6	1.64E-02	3.72E-23	8.42E-23
CpG25	17.7 ± 2.9	14.5 ± 2.5	21.2 ± 4.4	2.06E-11	2.23E-08	3.73E-21
CpG26	15.8 ± 3.0	14.8 ± 3.1	23.1 ± 6.3	9.83E-03	5.50E-16	3.81E-18
CpG27	11.0 ± 1.9	11.7 ± 2.5	19.8 ± 4.4	1.00E-02	2.30E-27	2.20E-24
CpG28	9.7 ± 2.9	10.5 ± 3.4	20.0 ± 7.3	1.43E-03	1.67E-23	2.22E-21
CpG29	15.9 ± 2.5	17.3 ± 3.2	28.4 ± 7.9	3.56E-04	1.23E-21	7.68E-19
CpG30	17.4 ± 3.2	17.6 ± 3.9	31.4 ± 8.3	7.38E-01	9.28E-24	1.37E-23
CpG31	21.1 ± 3.6	21.7 ± 4.0	35.2 ± 7.9	1.83E-01	6.00E-24	1.22E-22
Mean	14.9 ± 2.0	14.4 ± 1.9	22.0 ± 4.4	1.72E-02	1.41E-22	1.19E-24

Figure S1. CpG island within *LEP* gene proximal promoter region. The *LEP* exon 1 sequence is in bold type. The CpG sites are numbered according to Bouchard et al. 2010, and the underlined CpG sites were epigenotyped in the current study. DNA methylation levels at each CpG site were compared between blood, subcutaneous (SAT) and visceral (VAT) adipose tissues using the paired sample *t* test.



A

CpGs	3	4	5	6	7	11	12	13	14	15	16	17	23	24	25	26	27	28	29	30	31
3	1																				
4	0.63	1																			
5	0.63	0.85	1																		
6	0.78	0.86	0.88	1																	
7	0.74	0.77	0.84	0.91	1																
11	0.30	0.67	0.75	0.64	0.59	1															
12	0.47	0.54	0.59	0.54	0.54	0.63	1														
13	0.43	0.47	0.48	0.49	0.46	0.51	0.82	1													
14	0.51	0.50	0.62	0.61	0.55	0.65	0.69	0.67	1												
15	0.44	0.50	0.58	0.53	0.49	0.68	0.69	0.59	0.77	1											
16	0.24	0.49	0.39	0.47	0.38	0.64	0.34	0.32	0.52	0.57	1										
17	0.39	0.55	0.60	0.55	0.52	0.79	0.58	0.51	0.69	0.74	0.78	1									
23	0.18	0.39	0.31	0.36	0.30	0.55	0.18	0.17	0.39	0.39	0.69	0.66	1								
24	0.20	0.58	0.55	0.52	0.43	0.83	0.41	0.36	0.59	0.61	0.79	0.82	0.77	1							
25	0.35	0.48	0.56	0.57	0.52	0.75	0.48	0.40	0.62	0.57	0.56	0.69	0.64	0.77	1						
26	0.26	0.46	0.57	0.50	0.45	0.76	0.51	0.48	0.64	0.61	0.55	0.70	0.58	0.83	0.84	1					
27	0.32	0.52	0.62	0.51	0.54	0.71	0.45	0.35	0.60	0.62	0.45	0.63	0.61	0.71	0.77	0.77	1				
28	0.12	0.35	0.46	0.36	0.30	0.58	0.34	0.33	0.53	0.40	0.30	0.45	0.40	0.66	0.63	0.74	0.53	1			
29	0.14	0.49	0.44	0.42	0.31	0.70	0.43	0.40	0.48	0.58	0.67	0.70	0.61	0.87	0.68	0.79	0.62	0.61	1		
30	0.25	0.61	0.59	0.55	0.46	0.76	0.35	0.29	0.52	0.59	0.75	0.76	0.78	0.91	0.75	0.77	0.76	0.57	0.86	1	
31	0.15	0.48	0.45	0.41	0.32	0.70	0.27	0.20	0.48	0.56	0.71	0.69	0.70	0.89	0.68	0.76	0.70	0.58	0.84	0.89	1

B

CpGs	3	4	5	6	7	11	12	13	14	15	16	17	23	24	25	26	27	28	29	30	31
3	1																				
4	0.60	1																			
5	0.73	0.76	1																		
6	0.77	0.57	0.68	1																	
7	0.74	0.56	0.73	0.79	1																
11	0.54	0.68	0.65	0.63	0.51	1															
12	0.48	0.51	0.58	0.47	0.51	0.55	1														
13	0.25	0.20	0.39	0.20	0.39	0.14	0.45	1													
14	0.49	0.48	0.60	0.51	0.62	0.42	0.51	0.58	1												
15	0.37	0.28	0.49	0.38	0.45	0.36	0.51	0.63	0.63	1											
16	0.52	0.55	0.58	0.49	0.44	0.57	0.43	0.17	0.54	0.40	1										
17	0.38	0.59	0.55	0.56	0.44	0.65	0.42	0.25	0.60	0.50	0.62	1									
23	0.56	0.69	0.61	0.67	0.57	0.67	0.32	0.10	0.45	0.32	0.54	0.65	1								
24	0.59	0.73	0.75	0.63	0.58	0.69	0.47	0.36	0.60	0.54	0.65	0.70	0.79	1							
25	0.39	0.43	0.58	0.39	0.53	0.27	0.34	0.60	0.53	0.64	0.38	0.34	0.46	0.66	1						
26	0.63	0.66	0.71	0.72	0.66	0.68	0.50	0.30	0.52	0.42	0.57	0.59	0.75	0.79	0.60	1					
27	0.62	0.73	0.62	0.64	0.59	0.59	0.37	0.28	0.49	0.35	0.58	0.57	0.74	0.77	0.57	0.80	1				
28	0.32	0.25	0.42	0.41	0.34	0.35	0.25	0.01	0.22	0.11	0.21	0.24	0.48	0.40	0.17	0.53	0.27	1			
29	0.44	0.51	0.47	0.51	0.48	0.45	0.10	0.17	0.40	0.18	0.51	0.49	0.57	0.64	0.40	0.55	0.66	0.14	1		
30	0.55	0.66	0.61	0.57	0.53	0.64	0.35	0.25	0.46	0.32	0.66	0.62	0.70	0.77	0.47	0.70	0.76	0.32	0.80	1	
31	0.56	0.66	0.56	0.66	0.55	0.60	0.24	0.14	0.34	0.24	0.54	0.48	0.69	0.65	0.44	0.70	0.82	0.31	0.69	0.78	1

C

CpGs	3	4	5	6	7	11	12	13	14	15	16	17	23	24	25	26	27	28	29	30	31
3	1																				
4	0.22	1																			
5	0.28	0.67	1																		
6	0.55	0.38	0.58	1																	
7	0.65	0.35	0.42	0.62	1																
11	0.11	0.64	0.66	0.47	0.39	1															
12	0.48	0.42	0.40	0.36	0.55	0.37	1														
13	0.32	0.23	0.33	0.32	0.41	0.41	0.59	1													
14	0.44	0.40	0.54	0.47	0.42	0.50	0.53	0.51	1												
15	0.24	0.35	0.40	0.45	0.35	0.34	0.37	0.32	0.48	1											
16	0.17	0.48	0.46	0.44	0.28	0.60	0.28	0.17	0.44	0.43	1										
17	0.01	0.53	0.58	0.38	0.13	0.66	0.25	0.24	0.43	0.39	0.64	1									
23	0.30	0.63	0.55	0.42	0.52	0.70	0.45	0.31	0.39	0.29	0.50	0.49	1								
24	0.13	0.65	0.68	0.41	0.32	0.78	0.30	0.22	0.46	0.30	0.53	0.68	0.83	1							
25	0.33	0.55	0.61	0.44	0.45	0.59	0.42	0.23	0.39	0.29	0.35	0.43	0.77	0.76	1						
26	0.18	0.54	0.52	0.43	0.54	0.68	0.35	0.31	0.32	0.34	0.48	0.49	0.78	0.77	0.75	1					
27	0.01	0.61	0.55	0.23	0.24	0.73	0.20	0.13	0.30	0.14	0.43	0.57	0.65	0.73	0.59	0.71	1				
28	0.18	0.23	0.40	0.26	0.32	0.42	0.26	0.29	0.23	0.15	0.26	0.35	0.56	0.50	0.52	0.46	0.32	1			
29	0.12	0.57	0.58	0.41	0.36	0.75	0.23	0.17	0.36	0.17	0.47	0.57	0.64	0.74	0.63	0.69	0.73	0.33	1		
30	0.02	0.60	0.60	0.38	0.19	0.82	0.14	0.12	0.31	0.17	0.52	0.65	0.63	0.80	0.60	0.64	0.75	0.30	0.86	1	
31	0.02	0.58	0.51	0.29	0.13	0.71	0.20	0.15	0.29	0.12	0.55	0.55	0.50	0.64	0.52	0.57	0.65	0.29	0.68	0.78	1

Figure S2. Pearson correlation coefficients between CpGs at *LEP* proximal promoter locus in blood (**A**), subcutaneous (**B**), and visceral (**C**) adipose tissues.

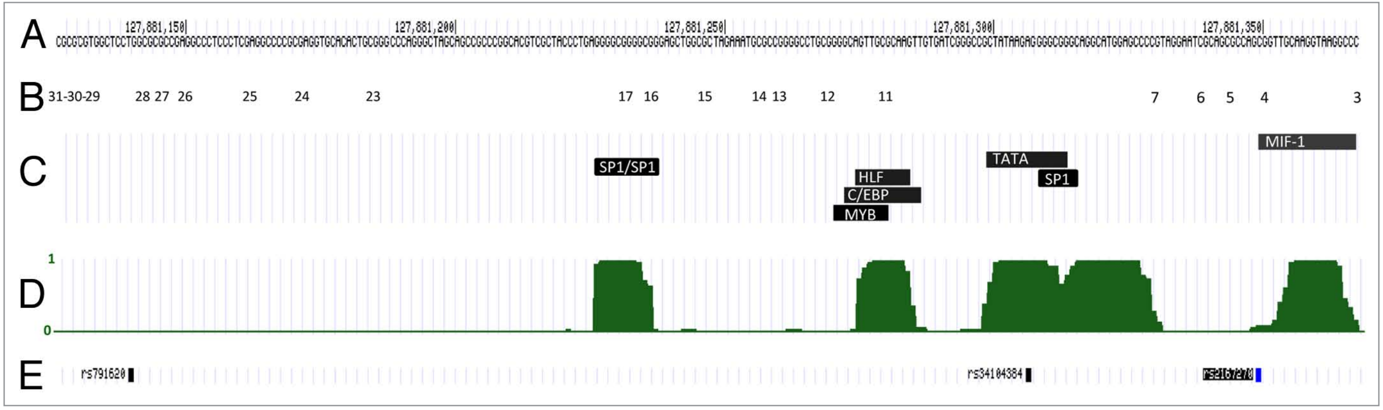


Figure S3. Location of transcription factor binding sites and highly conserved regions within the CpG island of the *LEP* gene proximal promoter region in placental mammals, adapted from UCSC genome browser tracks.[38] (**A**) *LEP* proximal promoter sequence. (**B**) Identification of epigenotyped CpG sites. (**C**) Localization of transcription factor binding sites. (**D**) PhastCons conservation track for placental mammals. (**E**) Single nucleotide polymorphisms (SNPs) previously identified in the *LEP* gene promoter. In blue, rs2167270 SNP analyzed in the current study. UCSC genome browser was accessed on September 19, 2013.

Table S2. Comparison of DNA methylation levels (%) at *LEP* proximal promoter CpG sites and LEP mRNA levels (AU) in SAT and VAT according to rs2167270 genotype (GG [*n* = 30]; GA/AA [*n* = 43]).

	SAT		VAT	
	GG	**GA/AA[a]**	**GG**	**GA/AA[a]**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
LEP-CpG3	33.5 ± 4.2	35.3 ± 5.7	34.2 ± 3.7	37.0 ± 5.0**
LEP-CpG4	20.1 ± 3.1	17.2 ± 3.3**	20.3 ± 3.3	17.7 ± 2.6**
LEP-CpG5	17.6 ± 2.9	17.4 ± 3.1	16.8 ± 3.3	17.1 ± 2.5
LEP-CpG6	15.5 ± 2.9	17.1 ± 3.9†	15.2 ± 2.9	17.3 ± 3.1**
LEP-CpG7	15.8 ± 2.7	16.9 ± 3.7	14.4 ± 2.3	15.9 ± 2.5*
LEP-CpG11	24.5 ± 3.0	24.4 ± 2.7	24.0 ± 4.3	23.8 ± 3.9
LEP-CpG12	8.7 ± 1.7	8.5 ± 1.4	7.6 ± 1.2	7.6 ± 1.3
LEP-CpG13	9.8 ± 1.4	9.7 ±1.9	6.8 ± 1.0	6.8 ± 1.4
LEP-CpG14	8.4 ± 1.3	8.8 ±1.5	5.9 ± 1.3	6.2 ± 1.1
LEP-CpG15	7.6 ± 1.1	7.8 ± 1.5	6.2 ± 1.0	6.3 ± 0.9
LEP-CpG16	6.3 ± 1.1	6.4 ± 1.4	5.7 ± 1.0	5.7 ± 1.3
LEP-CpG17	10.6 ± 2.0	10.7 ± 2.1	10.0 ± 2.3	9.9 ± 2.0
LEP-CpG23	8.7 ± 1.8	8.8 ± 1.8	9.3 ± 2.1	9.6 ± 1.9
LEP-CpG24	16.9 ± 2.4	16.8 ± 2.9	15.8 ± 3.7	16.0 ± 2.8
LEP-CpG25	17.9 ± 2.3	17.5 ± 3.3	14.3 ± 2.7	14.6 ± 2.5
LEP-CpG26	15.6 ± 2.9	15.9 ± 3.1	14.6 ± 3.3	14.9 ± 2.9
LEP-CpG27	11.2 ± 1.8	10.8 ± 1.9	11.7 ± 2.8	11.6 ± 2.2
LEP-CpG28	8.9 ± 3.5	10.2 ± 2.3†	9.8 ± 4.1	11.0 ± 2.8
LEP-CpG29	16.0 ± 2.3	15.9 ± 2.7	16.8 ± 3.6	17.6 ± 2.8
LEP-CpG30	17.4 ± 3.0	17.4 ± 3.3	17.7 ± 4.6	17.5 ± 3.3
LEP-CpG31	21.6 ± 3.7	20.7 ± 3.6	22.7 ± 4.1	21.0 ± 3.9†
LEP-Mean	14.9 ± 1.7	15.0 ± 2.1	14.3 ± 2.1	14.5 ± 1.7
LEP mRNA levels	1.02 ± 0.05	1.03 ± 0.04	0.97 ± 0.03	0.99 ± 0.03†

SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; AU, arbitrary units. Values are significantly different between GG and GA/AA genotypes: **$P < 0.01$; *$P < 0.05$; †$P < 0.10$.

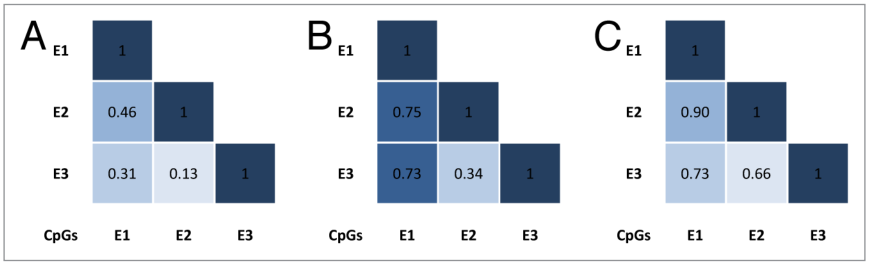
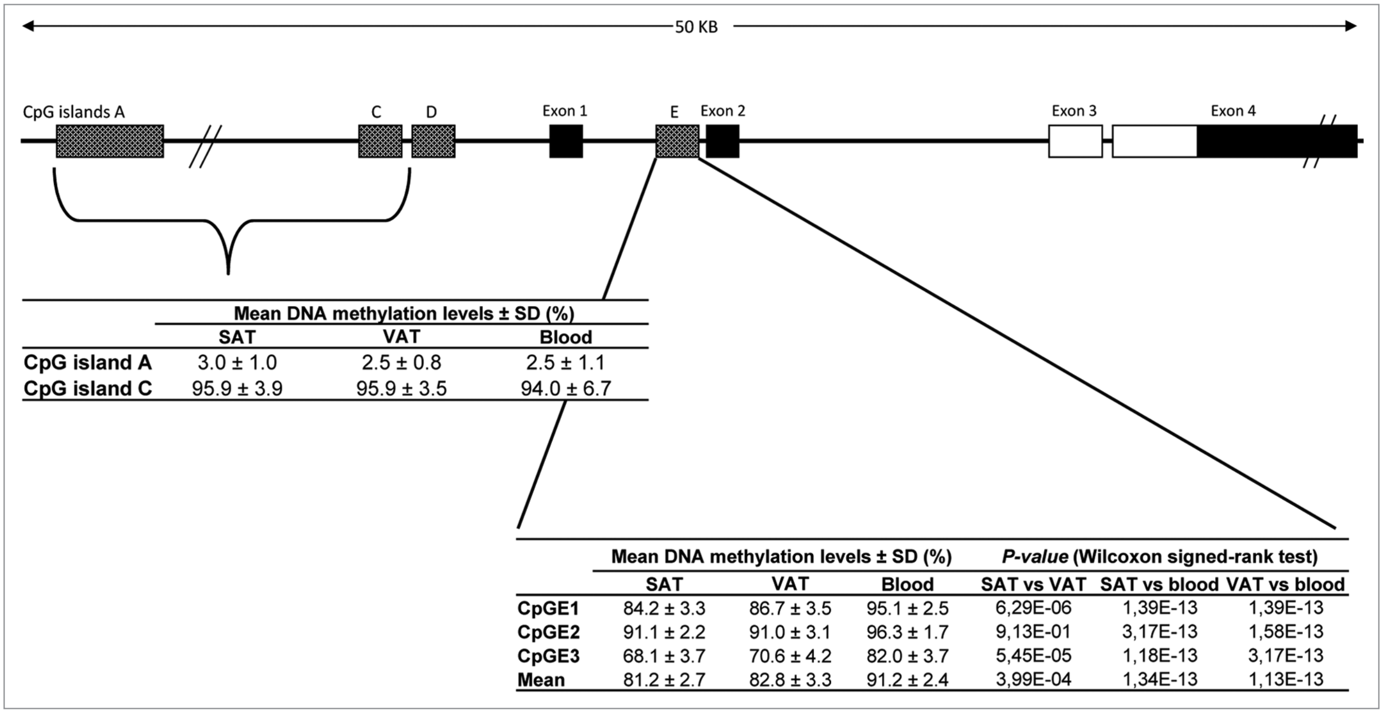


Figure S4. Spearman correlation coefficients between CpGs at *ADIPOQ* locus in blood (**A**), subcutaneous (**B**), and visceral (**C**) adipose tissues.

Table S3. PCR and pyrosequencing primers for LEP gene proximal promoter CpG island amplification and pyrosequencing.

Amplified region	PCR and pyrosequencing primers	Length (bp)
CpG3 to CpG7	F: 5′-GGGGAGGGTA GGTATGGAG-3′ R: 5′-CCCTACATCC CTCCTAACT-3′ Seq: 5′-GGGTAGGTAT GGAGT-3′	162 bp
CpG11 to CpG17	F: 5′-GAGTTTTTGG AGGGATATTA AGGA-3′ R: 5′-CAACTCCCCC CCCCCCCCTC AAAATAA-3′ Seq: 5′-GGGAGGTATT TAAGGG-3′	194 bp
CpG23 to CpG31	F: 5′-ATTTTAGGGA GGTATTTAAG GGTG-3′ R: 5′-ACCATTCCTA CCAAACTCCA TACCTACCC-3′ Seq: 5′-GGTAAGTAGT TATTTTGAGG G-3′	239 bp

F, forward; R, reverse; Seq, sequencing. These primers were designed from bisulfite converted sequence using Pyromark Assay Design software (version 2.0.1.15; Qiagen).



	Mean DNA methylation levels ± SD (%)		
	SAT	VAT	Blood
CpG island A	3.0 ± 1.0	2.5 ± 0.8	2.5 ± 1.1
CpG island C	95.9 ± 3.9	95.9 ± 3.5	94.0 ± 6.7

	Mean DNA methylation levels ± SD (%)			*P-value* (Wilcoxon signed-rank test)		
	SAT	VAT	Blood	SAT vs VAT	SAT vs blood	VAT vs blood
CpGE1	84.2 ± 3.3	86.7 ± 3.5	95.1 ± 2.5	6,29E-06	1,39E-13	1,39E-13
CpGE2	91.1 ± 2.2	91.0 ± 3.1	96.3 ± 1.7	9,13E-01	3,17E-13	1,58E-13
CpGE3	68.1 ± 3.7	70.6 ± 4.2	82.0 ± 3.7	5,45E-05	1,18E-13	3,17E-13
Mean	81.2 ± 2.7	82.8 ± 3.3	91.2 ± 2.4	3,99E-04	1,34E-13	1,13E-13

Figure S5. CpG islands analyzed within the *ADIPOQ* gene locus. CpG island A and C were found to be hypomethylated and hypermethylated respectively and were not analyzed. For CpG island E, DNA methylation levels at CpG sites were compared between blood, subcutaneous (SAT), and visceral (VAT) adipose tissues using the Wilcoxon signed-rank test.