

SUPPLEMENTARY DATA

**Prolonged Mild Sleep Restriction Changes Epigenome-
Wide DNA Methylation in Peripheral Blood Cells: A
Randomized Crossover Trial**

**Rocio Barragán, Christian K. Dye, Brooke Aggarwal, Sanja Jelic, Oscar Coltell, Dolores Corella,
Marie-Pierre St-Onge**

SUPPLEMENTARY DATA

Supplementary Table 1. Cell type estimates stratified by time point and intervention condition.

	Baseline AS	Baseline SR	Week 6 AS	Week 6 SR	<i>P</i>
Granulocytes	0.643±0.130	0.642±0.124	0.632±0.142	0.677±0.118	0.065
Natural Killer	0.060±0.025	0.064±0.032	0.062±0.028	0.061±0.032	0.832
B cells	0.052±0.029	0.052±0.026	0.052±0.026	0.048±0.025	0.319
CD4+ T	0.170±0.058	0.165±0.063	0.171±0.068	0.155±0.058	0.158
CD8+ T	0.070±0.051	0.073±0.050	0.074±0.056	0.063±0.044	0.210
Monocytes	0.089±0.033	0.090±0.029	0.092±0.033	0.083±0.029	0.130

AS: adequate sleep; SR: sleep restriction; P-value calculated for the comparison after the intervention (week 6). The units for cell type correspond to estimated cell fractions (unitless).

SUPPLEMENTARY DATA

Supplementary Table 2. Dietary intake and sleep timing stratified by time point and intervention condition.

	Baseline AS	Baseline SR	Week 6 AS	Week 6 SR	P
Energy (kcal)	1833.8±546.8	1849.8±595.0	1887.2±562.3	1912.3±620.0	0.829
Carbohydrates (g)	215.2±72.8	217.3±85.1	212.0±83.8	214.2±75.3	0.888
Fat (g)	74.6±32.0	78.5±31.6	81.4±26.2	81.3±34.5	0.989
Proteins (g)	75.7±26.7	72.8±27.2	79.0±27.6	78.8±30.9	0.978
Sleep onset (h:min)	23:43±1:05	23:43±1:05	23:32±1:01	00:42±0:56	<0.001
Waketime (h:min)	7:58±1:09	7:58±1:09	7:34±1:18	7:22±0:57	0.344

AS: adequate sleep; g: grams; h:min: hours:minutes; SR: sleep restriction; P-value calculated for the comparison after the intervention (week 6)

SUPPLEMENTARY DATA

Supplementary Table 3. Differentially methylated loci in the core clock selected candidate genes indicating changes in DNA that differ between sleep conditions (sleep restriction versus adequate sleep) ranked by p-value after multivariate adjustment.

CpG site	Gene Symbol	Chr: Position ¹	Genomic Location	Beta difference ²	<i>P</i> -interaction ³
cg02394126	ARNTL	chr11:13,357,482-13,357,483	TSS1500	0.005	0.00036
cg23506964	CLOCK	chr4:55,547,044-55,547,045	TSS1500	0.001	0.00136
cg13576304	RORA	chr15:60,644,877-60,644,878	Gene body	-0.002	0.00750
cg03701037	NPAS2	chr2:100,985,036-100,985,037	Gene body	0.001	0.00857
cg06606972	NPAS2	chr2:100,826,181-100,826,182	Intronic	0.009	0.00876
cg24416197	PER3	chr1:7,842,793-7,842,794	3'UTR	0.032	0.01375
cg05568797	BHLHE41	chr12:26,122,117-26,122,118	5'UTR	-0.002	0.01447
cg22952210	ARNTL2	chr12:27,332,783-27,332,784	TSS200	0.001	0.01669
cg14794298	ARNTL	chr11:13,315,127-13,315,128	Intronic	-0.002	0.01712
cg15113672	RORA	chr15:60,932,345-60,932,346	Intronic	0.015	0.01835
cg09176901	TIMELESS	chr12:56,424,843-56,424,844	Gene body	0.002	0.01865
cg02220837	BHLHE40	chr3:4,983,088-4,983,089	Gene body	0.003	0.01896
cg05826175	CLOCK	chr4:55435464-55435465	Gene body	-0.006	0.02114
cg08223837	CSNK1D	chr17:82,274,564-82,274,565	Gene body	0.013	0.02324
cg15675740	CRY2	chr11:45853066-45853067	Intronic	0.010	0.02380
cg12061096	RORA	chr15:60,736,042-60,736,043	TSS1500	-0.008	0.02419
cg10334778	CSNK1E	chr22:38,315,493-38,315,494	Intronic	-0.011	0.02450
cg22130371	RORA	chr15:60,628,418-60,628,419	TSS1500	0.001	0.02592
cg27280396	RORA	chr15:61,229,516-61,229,517	TSS1500	0.003	0.03261
cg16157435	NPAS2	chr2:100,819,089-100,819,090	Gene body	-0.004	0.03601
cg25773490	BHLHE40	chr3:4,979,000-4,979,001	TSS1500	-0.001	0.03670
cg07971790	RORA	chr15:60,643,931-60,643,932	Intronic	0.011	0.04290
cg03671396	PER2	chr2:238,258,282-238,258,283	Gene body	0.001	0.04340
cg25233914	ARNTL2	chr12:27,331,529-27,331,530	TSS1500	-0.006	0.04455
cg19986909	NPAS2	chr2:100,925,009-100,925,010	TSS200	0.014	0.04501
cg22074032	RORA	chr15:61,063,868-61,063,869	Intronic	0.017	0.04700
cg11569219	CLOCK	chr4:55,547,048-55,547,049	TSS1500	0.004	0.04732
cg21219996	RORB	chr9:74,498,154-74,498,155	Intronic	-0.003	0.04773

Models were adjusted for condition (sleep restriction or adequate sleep), phase, week (baseline or 6 weeks), age, body mass index and estimated leukocyte counts (B, NK, CD4+ T, CD8+ T, granulocytes and monocytes). ¹Position is expressed in values from the UCSC Genome Browser on Human (GRCh38/hg38). ²Beta difference indicates difference in methylation beta values (0 to 1) between conditions post intervention. ³*P*-interaction estimated for the condition x week interaction.

SUPPLEMENTARY DATA

Supplementary Table 4. P-values for the top 5 methylated CpGs (ranked by p-value) in the most significant core clock genes associated with sleep restriction. Uncorrected and after correction for multiple comparisons using the FDR after PCA.

Gene: CpG	CHR	Position ¹	P ²	RANK ³	P _{adjusted} ⁴	CpGs ⁵	PCA ⁶ factors
ARNTL:						58	19
cg02394126	11	13379029	0.0004	1	0.0069		
cg14794298	11	13336674	0.0171	2	0.1626		
cg07819357	11	13373854	0.0533	3	0.3373		
cg24231121	11	13342948	0.1020	4	0.4847		
cg21423556	11	13378933	0.1405	5	0.5321		
CLOCK:						38	11
cg23506964	4	56413211	0.0014	1	0.0150		
cg05826175	4	56301631	0.0211	2	0.1163		
cg11569219	4	56413215	0.0473	3	0.1456		
cg26535070	4	56325135	0.0530	4	0.1735		
cg08935434	4	56334911	0.1073	5	0.2360		
RORA:						180	47
cg13576304	15	60937076	0.0075	1	0.3526		
cg15113672	15	61224544	0.0183	2	0.3790		
cg12061096	15	61028241	0.0242	3	0.3790		
cg22130371	15	60920617	0.0259	4	0.4312		
cg27280396	15	61521715	0.0326	5	0.4312		
NPAS2						80	22
cg03701037	2	101601498	0.0086	1	0.0963		
cg06606972	2	101442643	0.0088	2	0.0963		
cg16157435	2	101435551	0.0360	3	0.2291		
cg19986909	2	101541471	0.0450	4	0.2476		
cg27273029	2	101435631	0.0521	5	0.2640		
PER3						31	9
cg24416197	1	7902853	0.0138	1	0.1238		
cg00811891	1	7904830	0.0756	2	0.3309		
cg00019458	1	7887942	0.1364	3	0.3403		
cg01444397	1	7844450	0.1471	4	0.4093		
cg20642605	1	7870707	0.1502	5	0.4093		
BHLHE41:						21	8
cg05568797	12	26275050	0.0145	1	0.1157		
cg16047471	12	26274563	0.1041	2	0.4163		
cg15398617	12	26277706	0.2239	3	0.5230		
cg21140419	12	26277982	0.2615	4	0.5230		
cg01728053	12	26278206	0.2679	5	0.5969		

Chr: chromosome. ¹Position is expressed in base-pair from the UCSC Genome Browser on Human (GRCh38/hg38). ²P-value unadjusted for the interaction term (condition x week interaction). ³RANK is the order used for p-value adjustment in the Benjamini-Hochberg procedure. ⁴P_{adjusted}: False discovery rate (FDR) adjusted for p-value. ⁵CpGs: Methylated loci. ⁶PCA: Principal Component Analysis. PCA factors is the number of selected components.

SUPPLEMENTARY DATA

Supplementary Table 5. The top 60 of methylated loci in the epigenome-wide study for the intervention trial for sleep condition (sleep restriction versus adequate sleep) ranked by p-value after multivariate adjustment.

CpG site	Gene Symbol	Chr: Position ¹	Genomic Location	Beta difference ²	<i>P</i> -interaction ³
cg23738833	SNHG3-RCC1	chr1:28,505,869-28,505,870	TSS200	-0.002	1.34 x 10 ⁻⁰⁶
cg13280380	FAF1	chr1:50,725,026-50,725,027	Gene body	0.012	2.25 x 10 ⁻⁰⁵
cg03179866	MMP12	chr11:102,875,904-102,875,905	TSS1500	0.002	2.78 x 10 ⁻⁰⁵
cg13063696	ZHX2	chr8:122,806,509-123,818,748	TSS1500	0.001	3.21 x 10 ⁻⁰⁵
cg03570498	MGMT	chr10:129,768,463-129,768,464	3'UTR	-0.001	3.26 x 10 ⁻⁰⁵
cg06292163	ANKRD44	chr2:197,187,514-197,187,515	Intronic	0.012	3.48 x 10 ⁻⁰⁵
cg06264879	NEU1	chr6:31,860,194-31,860,195	Gene body	0.002	3.51 x 10 ⁻⁰⁵
cg11088510	CENPBD1P	chr16:89,972,870-89,972,871	TSS1500	-0.003	3.62 x 10 ⁻⁰⁵
cg01192745		chr3:31,197,548-31,197,549	Intergenic	0.009	3.68 x 10 ⁻⁰⁵
cg15014826	ZSWIM4	chr19:13,833,633-13,833,634	Exon 1	-0.002	3.70 x 10 ⁻⁰⁵
cg16742090		chr16:55,033,738-55,033,739	Intergenic	0.002	3.94 x 10 ⁻⁰⁵
cg03118021	GMDS	chr6:1,837,921-1,837,922	Intronic	0.003	3.97 x 10 ⁻⁰⁵
cg20014778	URB2	chr1:229,625,867-229,625,868	TSS1500	-0.002	4.20 x 10 ⁻⁰⁵
cg22825220	UNC13C	chr15:54,290,247-54,290,248	Intronic	0.006	4.73 x 10 ⁻⁰⁵
cg26611683	FOXO1	chr13:40,667,597-40,667,598	TSS1500	-0.005	5.82 x 10 ⁻⁰⁵
cg14401496	DTHD1	chr4:36,332,653-36,332,654	TSS200	3.65 x 10 ⁻⁰⁴	6.01 x 10 ⁻⁰⁵
cg15993418		chr7:36,304,178-36,304,179	Intronic	-2.55 x 10 ⁻⁰⁴	6.05 x 10 ⁻⁰⁵
cg26234658		chr7:36,304,178-36,304,179	Intergenic	-2.93 x 10 ⁻⁰⁴	6.21 x 10 ⁻⁰⁵
cg13149641	ZNF623	chr8:143,635,530-143,635,531	TSS1500	-7.59 x 10 ⁻⁰⁴	6.23 x 10 ⁻⁰⁵
cg07240487	NPSR1	chr7:34,776,661-34,776,662	Intronic	8.19 x 10 ⁻⁰⁴	6.37 x 10 ⁻⁰⁵
cg09899430	KALRN	chr3:124,635,271-124,635,272	Intronic	8.62 x 10 ⁻⁰⁴	6.87 x 10 ⁻⁰⁵
cg23739646		chr15:43,167,808-43,167,809	Intergenic	0.002	7.19 x 10 ⁻⁰⁵
cg24342043		chr19:28,610,904-28,610,905	Intergenic	0.002	7.44 x 10 ⁻⁰⁵
cg14906281		chr10:34,994,792-35,283,720	Intergenic	3.23 x 10 ⁻⁰⁴	7.68 x 10 ⁻⁰⁵
cg08834787		chr17:45,374,026-45,374,027	Intergenic	-6.88 x 10 ⁻⁰⁴	7.78 x 10 ⁻⁰⁵
cg10548624	CEACAM7	chr19:41,691,535-41,691,536	Intronic	-2.54 x 10 ⁻⁰⁴	8.08 x 10 ⁻⁰⁵
cg26718574	MTPAP	chr10:30,349,205-30,349,206	Gene body	0.001	8.11 x 10 ⁻⁰⁵
cg21647189	PROM1	chr4:16,084,191-16,084,192	TSS1500	-7.46 x 10 ⁻⁰⁴	8.12 x 10 ⁻⁰⁵
cg17818344	ATCAY	chr19:3,880,608-3,880,609	TSS200	-5.10 x 10 ⁻⁰⁴	8.17 x 10 ⁻⁰⁵
cg01288184	CABLES1	chr18:23,231,444-23,231,445	Intronic	-4.83 x 10 ⁻⁰⁴	8.27 x 10 ⁻⁰⁵
cg01442682	RNU6-756P	chr8:124,260,739-125,272,980	Exon 1	-0.003	8.30 x 10 ⁻⁰⁵
cg21195843	SNORA60	chr20:38446584-38447211	TSS200	-1.08 x 10 ⁻⁰⁴	8.45 x 10 ⁻⁰⁵
cg27534424	XKR6	chr8:11,108,714-11,108,715	Gene body	-0.001	9.42 x 10 ⁻⁰⁵
cg06950683	CBFA2T3	chr16:88,944,392-88,944,393	Intronic	-0.016	9.96 x 10 ⁻⁰⁵
cg18458703	TRIOBP	chr22:37,732,316-37,732,317	Intronic	2.77 x 10 ⁻⁰⁴	1.00 x 10 ⁻⁰⁴

SUPPLEMENTARY DATA

cg01683948	ANKS3	chr16:4,734,195-4,734,196	5UTR	-1.74 x 10 ⁻⁰⁴	1.02 x 10 ⁻⁰⁴
cg14588411	SRI	chr7:88,221,062-88,221,063	TSS1500	-7.31 x 10 ⁻⁰⁴	1.06 x 10 ⁻⁰⁴
cg15317001	RSPO2	chr8:108,015,325-108,015,326	Intronic	0.002	1.07 x 10 ⁻⁰⁴
cg04714939	ZMIZ1	chr10:79,157,863-79,157,864	Intronic	-0.002	1.17 x 10 ⁻⁰⁴
cg14351775		chr9:131,115,058-133,990,445	Intergenic	-0.004	1.24 x 10 ⁻⁰⁴
cg21287449		chr16:24,445,929-24,445,930	Intergenic	-0.002	1.25 x 10 ⁻⁰⁴
cg19380984	TDRD3	chr13:60,501,075-60,501,076	Intronic	2.81 x 10 ⁻⁰⁴	1.26 x 10 ⁻⁰⁴
cg01212848	ABLIM1	chr10:114,604,611-114,604,612	Intronic	3.95 x 10 ⁻⁰⁵	1.26 x 10 ⁻⁰⁴
cg25172478		chr12:110,445,976-110,445,977	Intergenic	-0.002	1.35 x 10 ⁻⁰⁴
cg11976079	NECTIN1	chr11:119,655,594-119,655,595	TSS1500	0.002	1.37 x 10 ⁻⁰⁴
cg02577745	MIR493	chr14:100,868,245-100,868,246	TSS1500	0.004	1.40 x 10 ⁻⁰⁴
cg03138031		chr4:172,515,539-172,515,540	Intergenic	-9.09 x 10 ⁻⁰⁴	1.54 x 10 ⁻⁰⁴
cg08224415	SPRY1	chr4:123,397,112-123,397,113	TSS1500	-3.26 x 10 ⁻⁰⁴	1.54 x 10 ⁻⁰⁴
cg16885861	CD81	chr11:2,380,116-2,380,117	Intronic	0.004	1.58 x 10 ⁻⁰⁴
cg06790581	CRADD	chr12:93,683,368-93,683,369	Intronic	0.001	1.59 x 10 ⁻⁰⁴
cg09421620	FLI1	chr11:128,705,134-128,705,135	Intronic	-3.39 x 10 ⁻⁰⁴	1.60 x 10 ⁻⁰⁴
cg25681946		chr21:18,591,705-18,591,706	Intergenic	0.002	1.62 x 10 ⁻⁰⁴
cg20923877	IRF2	chr4:184,399,672-184,399,673	Intronic	6.50 x 10 ⁻⁰⁴	1.68 x 10 ⁻⁰⁴
cg22780070		chr17:61474589-61474590	Intergenic	0.004	1.70 x 10 ⁻⁰⁴
cg11189913	EDNRA	chr4:147,542,802-148,463,954	3'UTR	-0.003	1.74 x 10 ⁻⁰⁴
cg06921606	KIFC1	chr6:33,391,678-33,391,679	TSS200	7.64 x 10 ⁻⁰⁴	1.83 x 10 ⁻⁰⁴
cg24926041	TBC1D9	chr4:140,706,277-140,706,278	Intronic	1.16 x 10 ⁻⁰⁴	1.85 x 10 ⁻⁰⁴
cg16989274	ATXN2	chr12:111,599,677-111,599,678	TSS1500	-4.34 x 10 ⁻⁰⁴	1.85 x 10 ⁻⁰⁴
cg17274599		chr4:118,400,619-118,400,620	Intergenic	-0.001	1.86 x 10 ⁻⁰⁴
cg05300414	SNORD36A	chr9:133,350,448-133,350,449	TSS200	1.86 x 10 ⁻⁰⁴	1.92 x 10 ⁻⁰⁴

Models were adjusted for condition (sleep restriction or adequate sleep), phase, week (baseline or follow-up), age, body mass index, and cell type composition (B, NK, CD4+ T, CD8+ T, neutrophil and monocytes). ¹Position is expressed in values from the UCSC Genome Browser on Human (GRCh38/hg38). ²Beta difference indicates difference in methylation beta values (0 to 1) between conditions post intervention. ³*P*-interaction estimated for the condition x week interaction. Chr: chromosome.

SUPPLEMENTARY DATA

Supplementary Table 6. KEGG pathway enrichment and GO biological functions identified through epigenome-wide methylation profiling related to sleep restriction. Uncorrected p-values are presented on the table.

KEGG Pathways Term	<i>P</i>
TGF-beta signaling pathway	0.0166
Fatty acid degradation	0.0233
Glucagon signaling pathway	0.0250
Amino sugar and nucleotide sugar metabolism	0.0306
Synaptic vesicle cycle	0.0731
Thiamine metabolism	0.0822
Transcriptional misregulation in cancer	0.0971
Other glycan degradation	0.0978
Prostate cancer	0.1059
Focal adhesion	0.1075
Steroid biosynthesis	0.1081
Insulin resistance	0.1263

GO Biological Process Term	<i>P</i>
Long-Chain Fatty Acid Metabolic Process (GO:0001676)	0.0010
Calcium Ion-Regulated Exocytosis Of Neurotransmitter (GO:0048791)	0.0028
Protein Acetylation (GO:0006473)	0.0118
Immunoglobulin Mediated Immune Response (GO:0016064)	0.0126
Regulation Of Amyloid-Beta Formation (GO:1902003)	0.0152
Positive Regulation Of Protein Catabolic Process (GO:0045732)	0.0157
Negative Regulation Of Amide Metabolic Process (GO:0034249)	0.0161
Regulation Of Protein Metabolic Process (GO:0051246)	0.0176
Protein Acylation (GO:0043543)	0.0188
Response To Transforming Growth Factor Beta (GO:0071559)	0.0198
Negative Regulation Of Protein Secretion (GO:0050709)	0.0208
Synaptic Vesicle Endocytosis (GO:0048488)	0.0218
Negative Regulation Of MAPK Cascade (GO:0043409)	0.0222
Synaptic Vesicle Exocytosis (GO:0016079)	0.0229
Regulation Of Amide Metabolic Process (GO:0034248)	0.0229
Positive Regulation Of T Cell Activation (GO:0050870)	0.0233
Positive Regulation Of Wnt Signaling Pathway (GO:0030177)	0.0245
Negative Regulation Of Lens Fiber Cell Differentiation (GO:1902747)	0.0282
Regulation Of Endothelial Cell-Matrix Adhesion Via Fibronectin (GO:1904904)	0.0282
GDP-mannose Metabolic Process (GO:0019673)	0.0282
Nucleoside Triphosphate Biosynthetic Process (GO:0009142)	0.0282
Cellular Response To Cold (GO:0070417)	0.0282
Podocyte Cell Migration (GO:0090521)	0.0282

SUPPLEMENTARY DATA

Renal Filtration Cell Differentiation (GO:0061318)	0.0282
Positive Regulation Of Wnt Signaling Pathway, Planar Cell Polarity Pathway (GO:2000096)	0.0282
Positive Regulation Of Activin Receptor Signaling Pathway (GO:0032927)	0.0282
Response To Angiotensin (GO:1990776)	0.0282
Positive Regulation Of Epithelial Cell Differentiation Involved In Kidney Development (GO:2000698)	0.0282
Establishment Of Lymphocyte Polarity (GO:0001767)	0.0282
Establishment Of Protein Localization To Telomere (GO:0070200)	0.0282
Subtelomeric Heterochromatin Formation (GO:0031509)	0.0282
Glomerular Epithelial Cell Differentiation (GO:0072311)	0.0282
Humoral Immune Response Mediated By Circulating Immunoglobulin (GO:0002455)	0.0282
Presynaptic Active Zone Organization (GO:1990709)	0.0282
Negative Regulation Of Cellular Metabolic Process (GO:0031324)	0.0283
Insulin Receptor Signaling Pathway (GO:0008286)	0.0295
Unsaturated Fatty Acid Metabolic Process (GO:0033559)	0.0318
Negative Regulation Of Heart Rate (GO:0010459)	0.0337
Negative Regulation Of Membrane Protein Ectodomain Proteolysis (GO:0051045)	0.0337
Regulation Of Lens Fiber Cell Differentiation (GO:1902746)	0.0337
Negative Regulation Of Retinoic Acid Receptor Signaling Pathway (GO:0048387)	0.0337
Negative Regulation Of Stress-Activated Protein Kinase Signaling Cascade (GO:0070303)	0.0337
Amino-Acid Betaine Metabolic Process (GO:0006577)	0.0337
Regulation Of Myosin-Light-Chain-Phosphatase Activity (GO:0035507)	0.0337
Neurotrophin TRK Receptor Signaling Pathway (GO:0048011)	0.0337
Regulation Of Relaxation Of Muscle (GO:1901077)	0.0337
Cellular Response To Hepatocyte Growth Factor Stimulus (GO:0035729)	0.0337
Positive Regulation Of Amyloid-Beta Clearance (GO:1900223)	0.0337
Response To Hepatocyte Growth Factor (GO:0035728)	0.0337
Synaptic Vesicle Docking (GO:0016081)	0.0337
Fatty Acid Elongation, Monounsaturated Fatty Acid (GO:0034625)	0.0337
Fatty Acid Elongation, Polyunsaturated Fatty Acid (GO:0034626)	0.0337
Fatty Acid Elongation, Saturated Fatty Acid (GO:0019367)	0.0337
Fatty Acid Elongation, Unsaturated Fatty Acid (GO:0019368)	0.0337
Ganglioside Catabolic Process (GO:0006689)	0.0337
Hemostasis (GO:0007599)	0.0337
Intracellular Sequestering Of Iron Ion (GO:0006880)	0.0337
Positive Regulation Of Triglyceride Catabolic Process (GO:0010898)	0.0337
Receptor-Mediated Virion Attachment To Host Cell (GO:0046813)	0.0337
Regulation Of Adiponectin Secretion (GO:0070163)	0.0337
Regulation Of Cardiac Muscle Hypertrophy In Response To Stress (GO:1903242)	0.0337
Regulation Of Release Of Sequestered Calcium Ion Into Cytosol (GO:0051279)	0.0343

SUPPLEMENTARY DATA

Fatty Acid Oxidation (GO:0019395)	0.0355
Regulation Of Exocytosis (GO:0017157)	0.0380
DNA Ligation (GO:0006266)	0.0392
Aldehyde Catabolic Process (GO:0046185)	0.0392
Regulation Of Mesenchymal Stem Cell Differentiation (GO:2000739)	0.0392
Neuron Projection Arborization (GO:0140058)	0.0392
Nucleobase-Containing Small Molecule Interconversion (GO:0015949)	0.0392
Regulation Of Protein Localization To Nucleolus (GO:1904749)	0.0392
Cellular Response To Nitric Oxide (GO:0071732)	0.0392
Cellular Response To Reactive Nitrogen Species (GO:1902170)	0.0392
Cytoplasmic Sequestering Of NF-kappaB (GO:0007253)	0.0392
siRNA Processing (GO:0030422)	0.0392
Positive Regulation Of Gluconeogenesis (GO:0045722)	0.0392
Store-Operated Calcium Entry (GO:0002115)	0.0392
Terpenoid Metabolic Process (GO:0006721)	0.0392
Immunological Synapse Formation (GO:0001771)	0.0392
Protein Localization To Cytoplasmic Stress Granule (GO:1903608)	0.0392
Myoblast Migration (GO:0051451)	0.0392
Regulation Of Endothelial Cell Development (GO:1901550)	0.0447
UDP-N-acetylglucosamine Biosynthetic Process (GO:0006048)	0.0447
Regulation Of Macrophage Migration (GO:1905521)	0.0447
Regulation Of Nephron Tubule Epithelial Cell Differentiation (GO:0072182)	0.0447
Peroxisomal Transport (GO:0043574)	0.0447
Positive Regulation Of Adaptive Immune Response (GO:0002821)	0.0447
Constitutive Heterochromatin Formation (GO:0140719)	0.0447
Positive Regulation Of Calcium Ion-Dependent Exocytosis (GO:0045956)	0.0447
Positive Regulation Of Epithelial Cell Proliferation Involved In Wound Healing (GO:0060054)	0.0447
Regulation Of Wnt Signaling Pathway, Planar Cell Polarity Pathway (GO:2000095)	0.0447

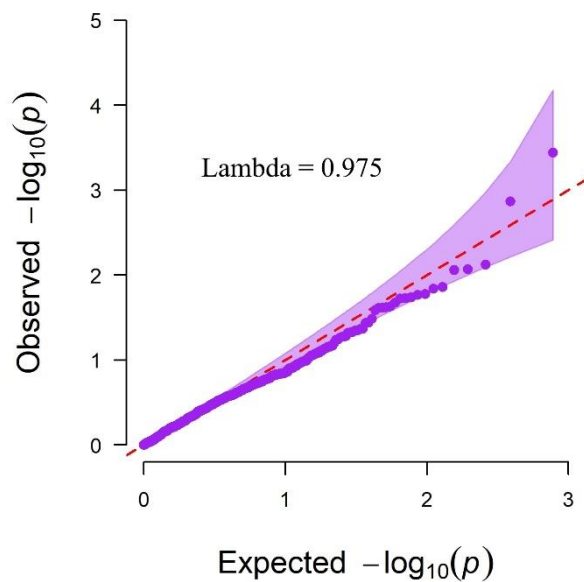
SUPPLEMENTARY DATA

Supplementary Table 7. Genes included in the most significant KEGG pathways and GO biological functions, detected in the epigenome-wide methylation profiling related to sleep restriction. Unadjusted p-values and related genes are presented in the table.

KEGG Pathways Term	Unadjusted p	Related genes
TGF-beta signaling pathway	0.0166	CREBBP;ROCK1;ACVR2A
Fatty acid degradation	0.0233	CPT1A;CREBBP;FOXO1
Glucagon signaling pathway	0.0250	CPT1A;ADH5
Amino sugar and nucleotide sugar metabolism	0.0306	GMDS;UAP1L1

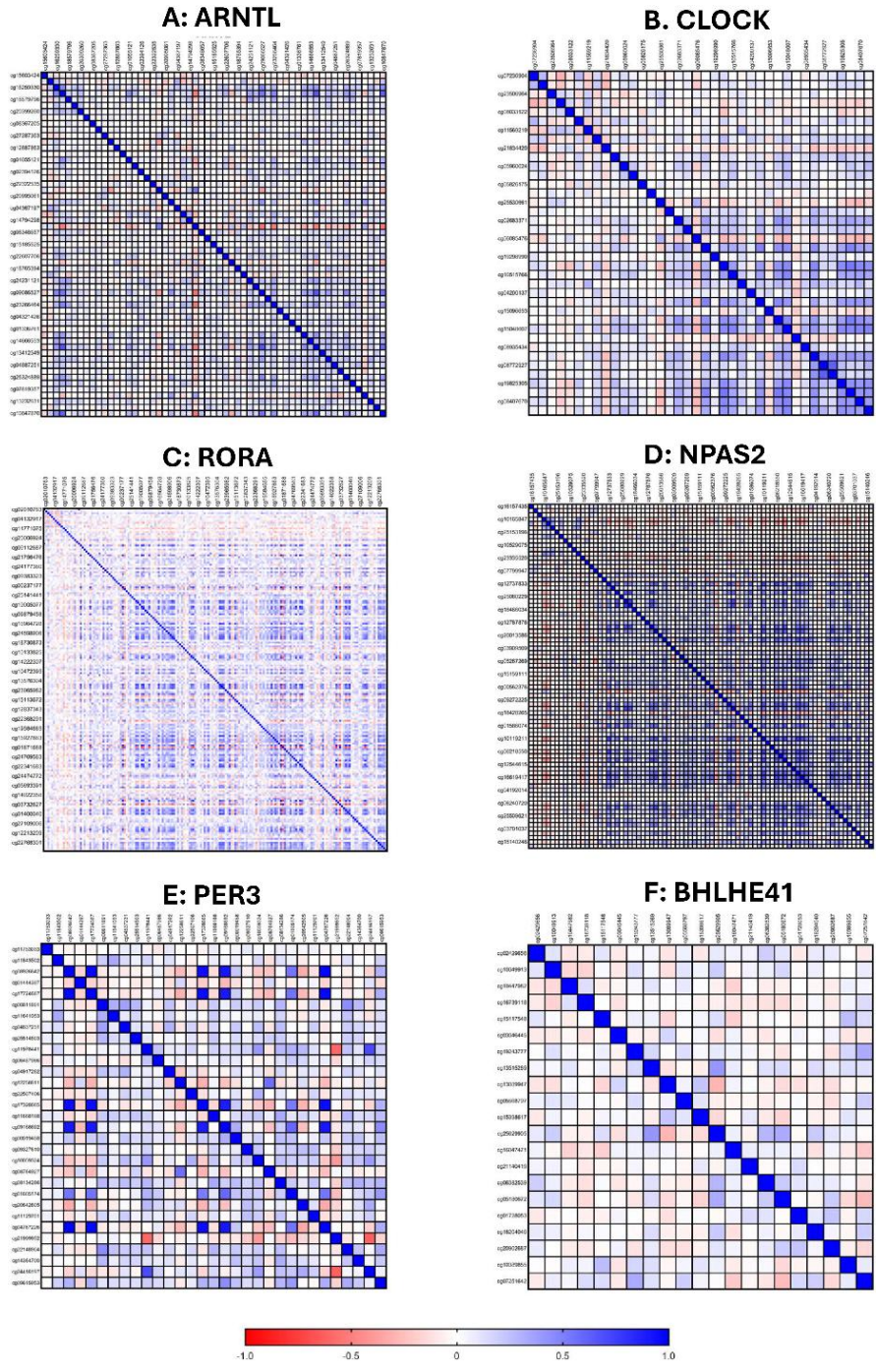
GO Biological Process Term	Unadjusted p	Related genes
Long-Chain Fatty Acid Metabolic Process (GO:0001676)	0.0010	CPT1A;ELOVL3;HACL1;DAGLB
Calcium Ion-Regulated Exocytosis Of Neurotransmitter (GO:0048791)	0.0028	UNC13C;SYT1
Protein Acetylation (GO:0006473)	0.0118	CREBBP;FOXO1
Immunoglobulin Mediated Immune Response (GO:0016064)	0.0126	CD81;HLA-DOA
Regulation Of Amyloid-Beta Formation (GO:1902003)	0.0152	ROCK1;GSAP
Positive Regulation Of Protein Catabolic Process (GO:0045732)	0.0157	CD81;FAF1;FOXO1
Negative Regulation Of Amide Metabolic Process (GO:0034249)	0.0161	TIA1;ATCAY;ROCK1
Regulation Of Protein Metabolic Process (GO:0051246)	0.0176	ATXN2;FAF1;GSAP
Protein Acylation (GO:0043543)	0.0188	CREBBP;FOXO1
Response To Transforming Growth Factor Beta (GO:0071559)	0.0198	ROCK1;SOX5

SUPPLEMENTARY DATA



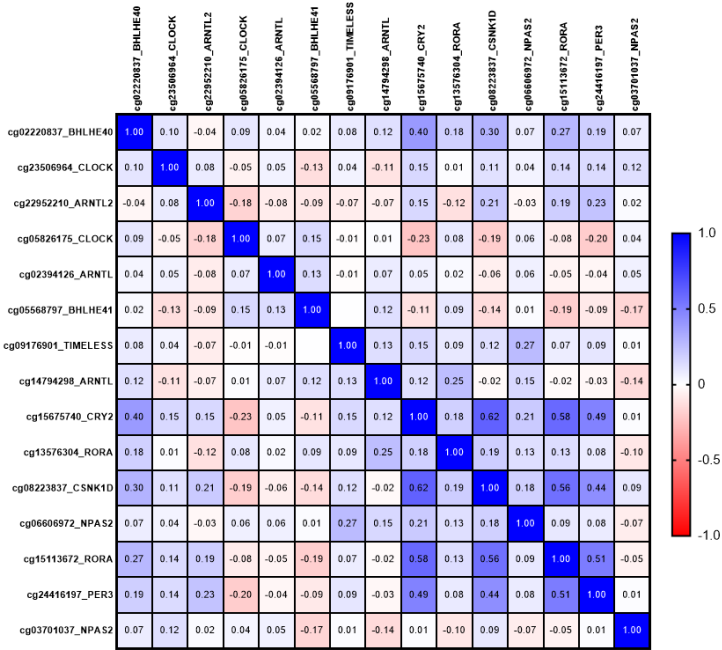
Supplementary Figure 1. Quantile-Quantile (QQ) plot for the CpG-site association for the selected candidate clock genes (777 CpGs), and sleep restriction. Model for repeated measures adjusted for sex, age, BMI, cell counts, phase, condition and week. P-values for the interaction term (condition * week). The corresponding lambda was computed.

SUPPLEMENTARY DATA



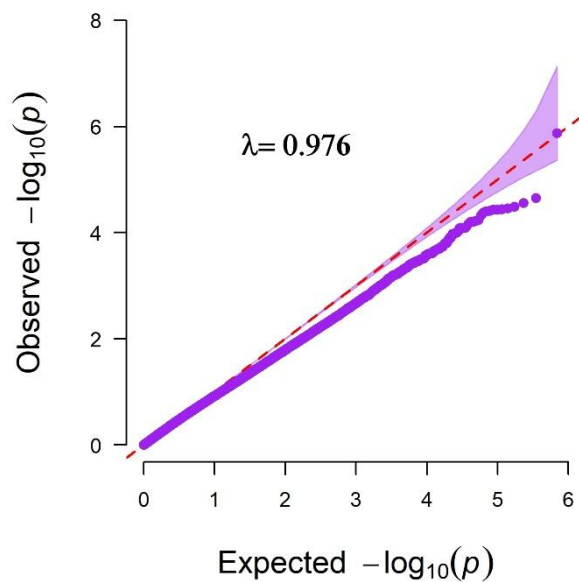
Supplementary Figure 2. Heatmaps for the correlation (Spearman coefficients) among all the CpGs (methylation levels) in the selected candidate genes (A: ARNTL; B: CLOCK; C: RORA; D: NPAS2; E: PER3; F: BHLHE41) in the participants at baseline.

SUPPLEMENTARY DATA



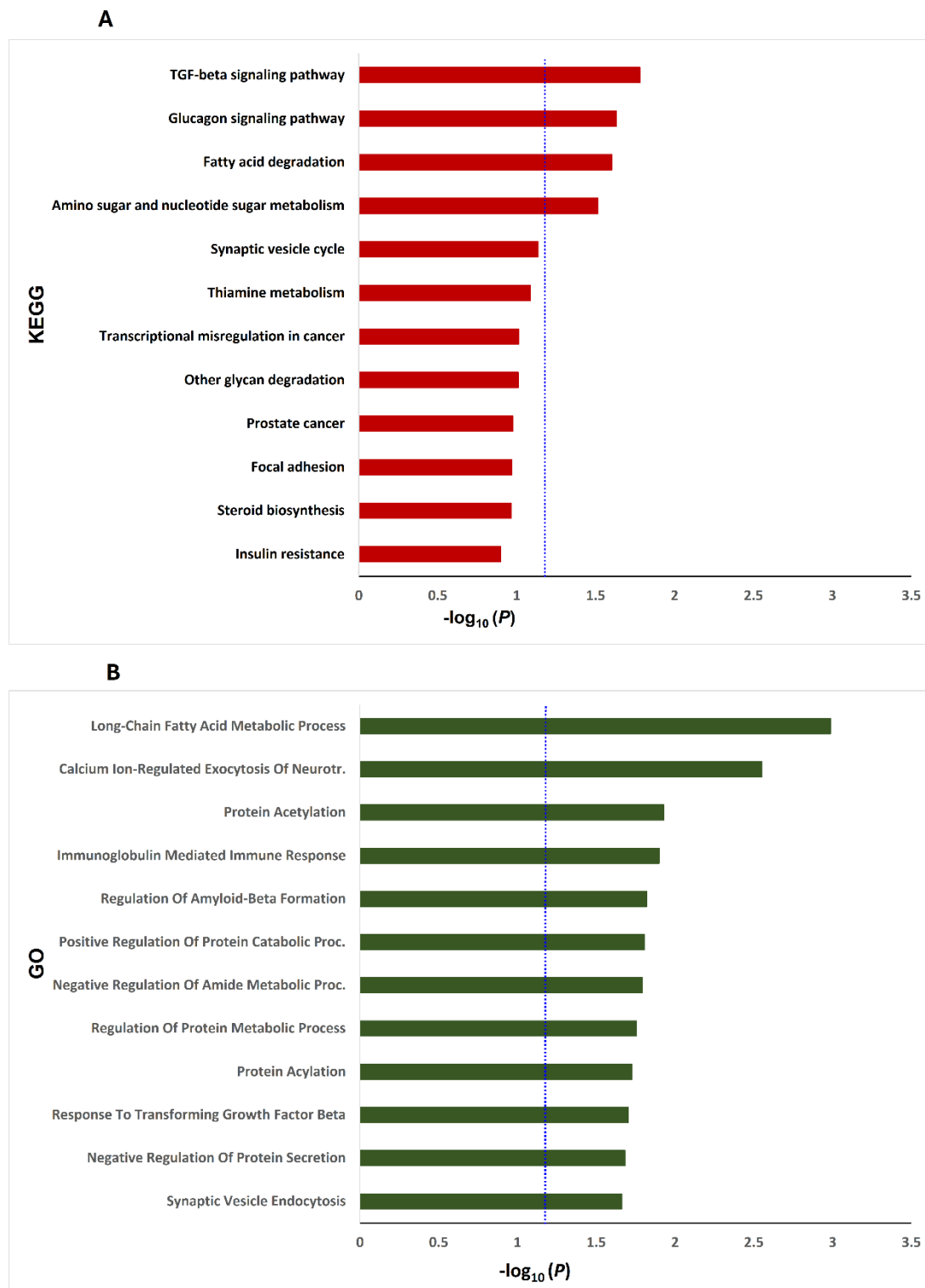
Supplementary Figure 3. Heatmap for the correlation (Spearman coefficients) among the top 15 CpGs differentially methylated in the selected candidate clock genes (Table 2) in the participants at baseline.

SUPPLEMENTARY DATA



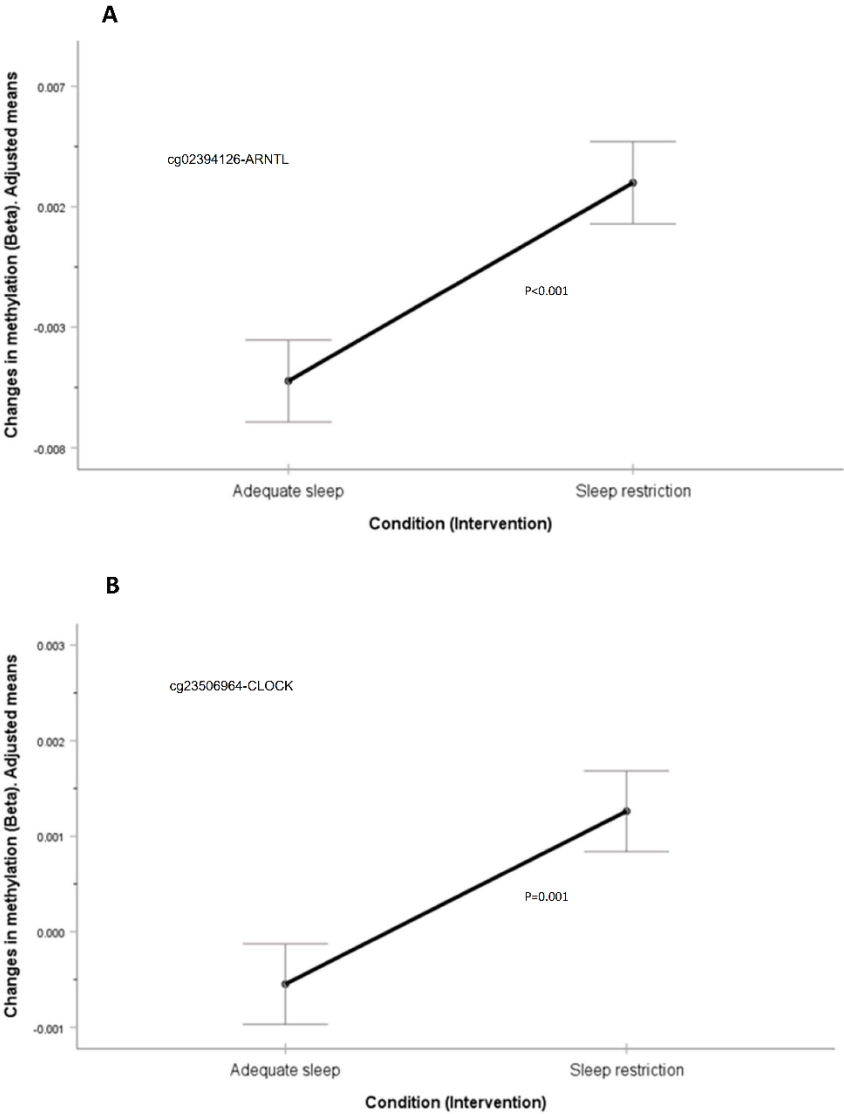
Supplementary Figure 4. Quantile-Quantile (QQ) plot for the CpG-site association in the EWAS for sleep restriction. Model for repeated measures adjusted for sex, age, BMI, cell counts, phase, condition and week. P-values for the interaction term (condition * week). The corresponding lambda was computed.

SUPPLEMENTARY DATA



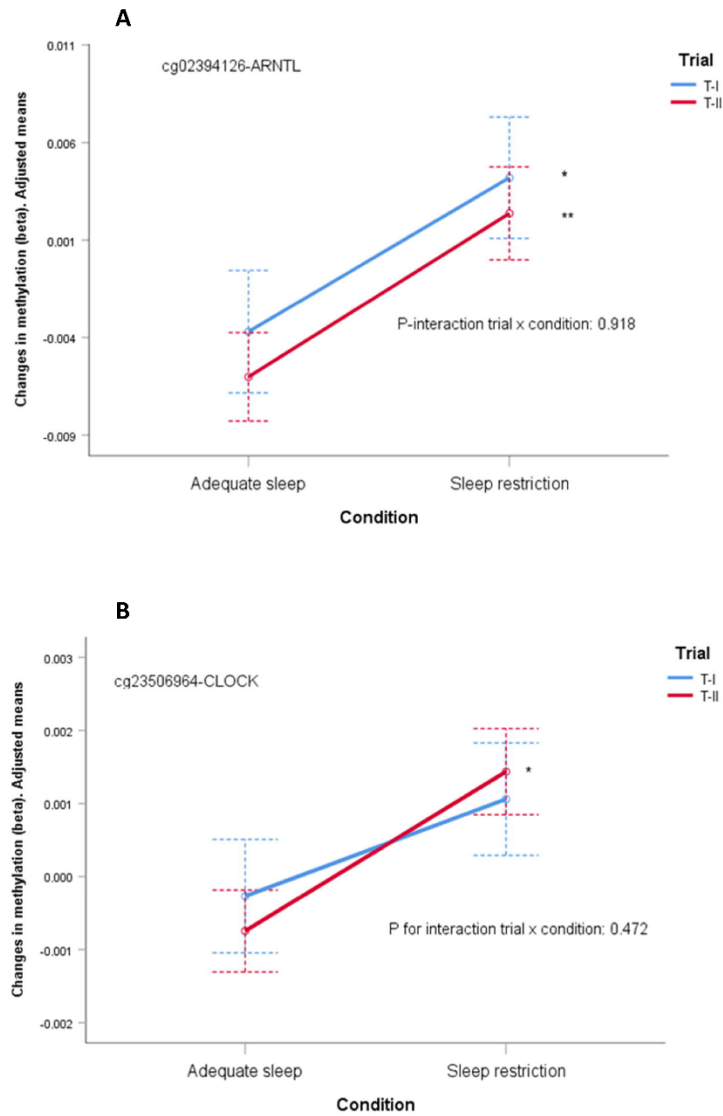
Supplementary Figure 5. The top 12 enriched KEGG pathways (A), and the top 12 GO enriched items in biological process and cellular component (B) from the differentially methylated loci in the EWAS for sleep restriction. The blue line represents nominally significant p-value ($p < 0.05$).

SUPPLEMENTARY DATA



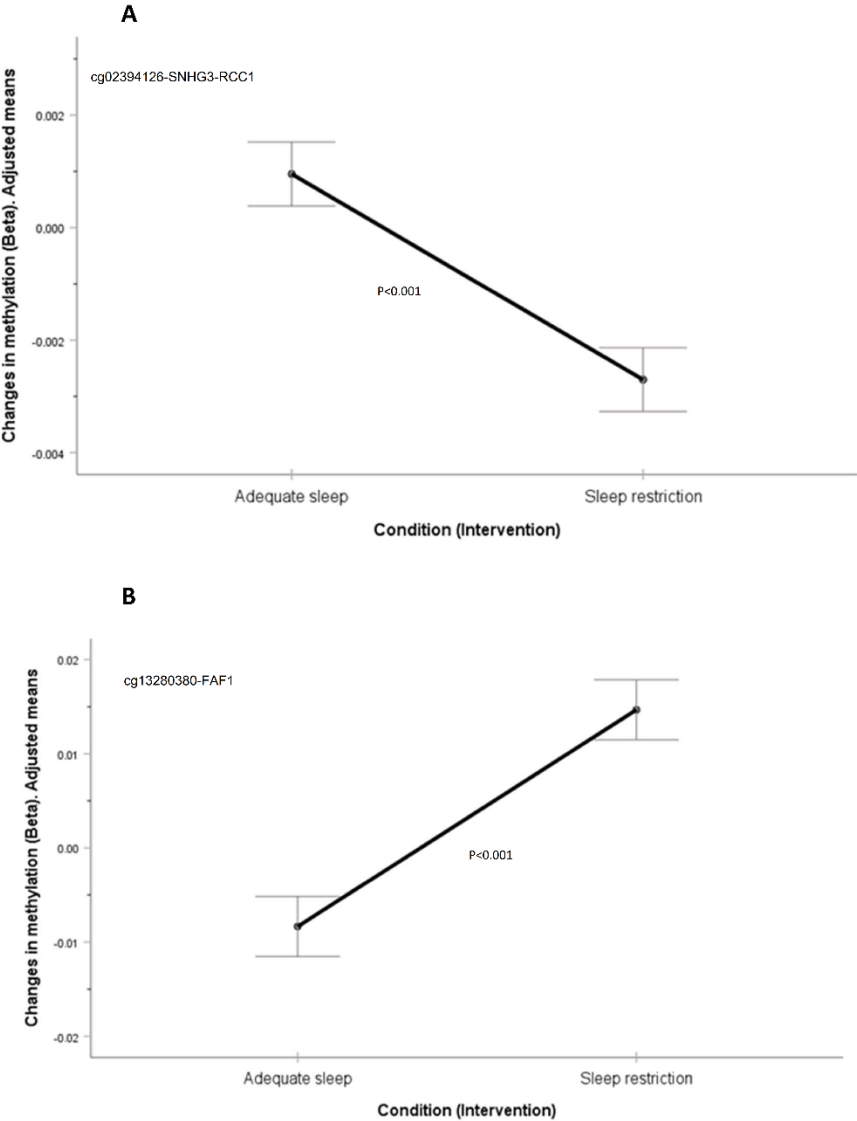
Supplementary Figure 6. Changes in methylation (beta-values) in the cg02394126-ARNTL (A), and the cg23506964-CLOCK (B), after 6-weeks of intervention depending on the condition (adequate sleep or sleep restriction). Multivariable model adjusted for sex, age, phase, BMI, and cell type composition. (Error bars: SE of means).

SUPPLEMENTARY DATA



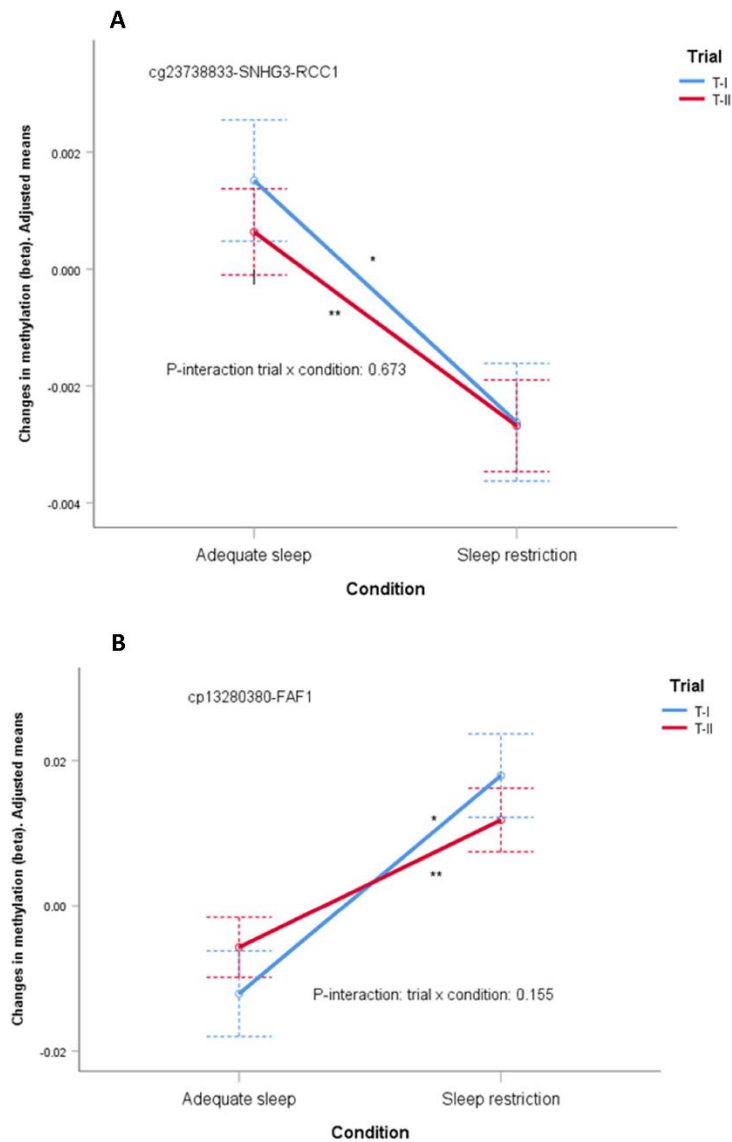
Supplementary Figure 7. Consistent effect of changes in methylation (beta-values) for the in the cg02394126-ARNTL (A), and the cg23506964-CLOCK (B), after 6-weeks of intervention depending on the condition (adequate sleep or sleep restriction), and the trials (T-I: NCT02835261; T-II: NCT02960776). Multivariable model adjusted for sex, age, phase, BMI, trial, and cell type composition. (Error bars: SE of means). P-interaction: Trial x condition for each CpG and stars indicate a significant condition effect ($p < 0.05$) in both trials (*= T-I; **= T-II).

SUPPLEMENTARY DATA



Supplementary Figure 8. Changes in methylation (beta-values) in the cg23738833_SNHG3-RCC1 (A), and the cg13280380-FAF1 (B), after 6-weeks of intervention depending on the condition (adequate sleep or sleep restriction). Multivariable model adjusted for sex, age, phase, BMI, and cell type composition. (Error bars: SE of means).

SUPPLEMENTARY DATA



Supplementary Figure 9. Consistent effect of changes in methylation (beta-values) for the in the cg23738833-SNHG3-RCC1 (A), and the cg13280380-FAF1 (B), after 6-weeks of intervention depending on the condition (adequate sleep or sleep restriction), and the trials (T-I: NCT02835261; T-II: NCT02960776). Multivariable model adjusted for sex, age, phase, BMI, trial, and cell type composition. (Error bars: SE of means). P-interaction: Trial x condition for each CpG and stars indicate a significant condition effect ($p < 0.05$) in both trials (*= T-I; **= T-II).

SUPPLEMENTARY DATA