Supporting Information

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SI Methods

Constructing and Visualizing Direct Interaction Networks. Direct interaction networks for a given gene list were constructed using the direct interaction network tool of MetaCore. Direct interaction networks were constructed for: (*i*) genes found to have a significant (corrected *P* value, <0.05) ANOVA main effect; and (*ii*) genes found to have a significant ANOVA interaction with a corrected $P < 0.02$. This lowering of the *P* value threshold was applied because of the large number of transcripts found to have an interaction and the lower limits of the MetaCore tool. The constructed networks were saved and exported to Cytoscape for visualization using the MetaCore Cytoscape plugin. Additional metainformation to be represented in the network (e.g., processes, connections, etc.) was collated using ad hoc Perl scripts.

SP1 Transcription Factor Binding Site Enrichment. Specificity protein (SP)1-binding sites in four human cell lines [B-lymphoblastoid cells (GM12878), hepatocellular liver carcinoma cells (HepG2), human embryonic stem cells (H1-hESC), and erythrocytic leukemia cells (K562)] were obtained from the Encyclopedia of DNA Elements (ENCODE) Uniform ChIP-seq dataset via the UCSC genome browser (accessed June 7, 2013). This dataset comprises the SP1 binding site regions identified following the ENCODE data analysis pipeline. To identify gene targets of SP1, the coordinates of SP1 binding were compared with the 1,000 bp upstream regions of all genes targeted by our array data that have annotated 5′ and 3′ UTRs (obtained from UCSC June 7, 2013). Those genes that had an SP1 binding site within their 1,000 bp upstream region were classed as an SP1 target in the respective cell line. For each cell line, the SP1-binding enrichment of the ANOVA interaction gene list, a gene list in which SP1 appears as a central hub of the direct interaction network constructed from those transcripts with an ANOVA corrected *P* value of <0.02, was calculated as the percentage of how many of the gene list were within the SP1 target list. To assess the significance of the SP1 binding site enrichment, 1,000 random gene lists, the same size as that of the ANOVA interaction list, were generated and used as the background distribution for performing a one-sample *t* test.

Contribution of the Sleep–Wake Cycle and Circadian Rhythmicity to the Time Course of Transcripts. To describe the expression profile of each transcript as a linear combination of the 28-h sleep–wake cycle and 24-h circadian rhythmicity, a linear model of the form $X_{pi} = a_p M_i + b_p S_i$, where X_{pi} is the median expression profile of transcript p at sampling point i, M_i is the melatonin profile at sampling point i, and S_i is the sleep profile at sampling point i, was fitted to the median expression profile (median across all participants, per sampling time point, of z-scored time-series) of each transcript using “lm” function in R (1). Melatonin and sleep profiles were created for each sleep condition (sleeping in phase and out of phase with melatonin) using sine waves with matching phases. The period for the melatonin profile was set to 24 h, whereas the period for sleep was set at 28 h. Coefficient estimates (a_p and b_p) and their associated SEs (SEa_p and SEb_p) of transcripts with an $R^2 > 0.6$ (n = 1,792 out of 41,119 transcripts) were used to classify transcripts into distinct categories based on the contribution of the 28-h sleep–wake cycle and 24-h circadian rhythmicity.

Comparison of ANOVA “Sleep Condition” and “Sample” Interaction Gene List Overlap with Gene Lists for Known CIRBP and RBM3 Binding. The “phyper” function within R was used to calculate the *P* value for a particular gene-list overlap based on the hypergeometric distribution. Here, the number of genes within a list [ANOVA interaction, cold-inducible RNA-binding protein (CIRBP), RNA-binding motif protein 3 (RBM3), and/or “background” comprising genes targeted by the array] was the number of genes within the original list [this work, data from Liu et al. (2), and the array probes used in this study] that were homologous between mouse and human, as determined through the MADGene tool.

1. Fox J, Weisberg S (2011) *An R Companion to Applied Regression* (Sage Publications, Thousand Oaks, CA).
2. Liu Y, et al. (2013) Cold-induced RNA-binding proteins regulate circadian gene expression by controlling alternative polyadenylation. *Sci Rep* 3:2054.

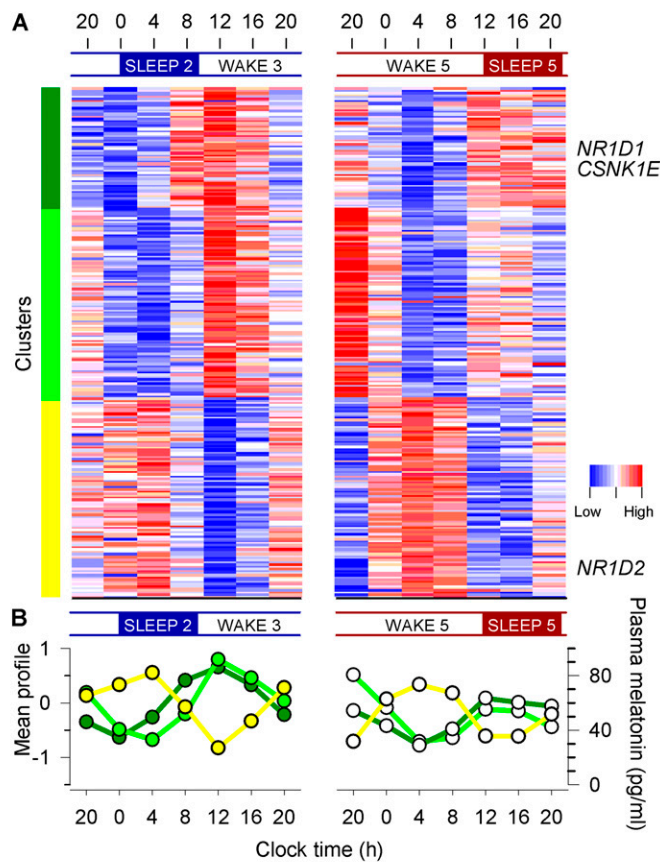

Fig. S1. Circadian transcripts when sleeping out of phase. (*A*) Median expression profiles (median of z-scored data across 19 paired participants per time point) of transcripts classified as circadian when sleeping out of phase with melatonin (right side) and their profiles when sleeping in phase (left side), clustered as indicated on the left with transcript examples annotated on the right. (*B*) Mean expression profiles for clusters of day (light and dark green) and night (yellow) transcripts while sleeping in phase (*Left*) with melatonin (blue curve) and out of phase (*Right*) with melatonin (pink curve) (n = 19 paired subjects).

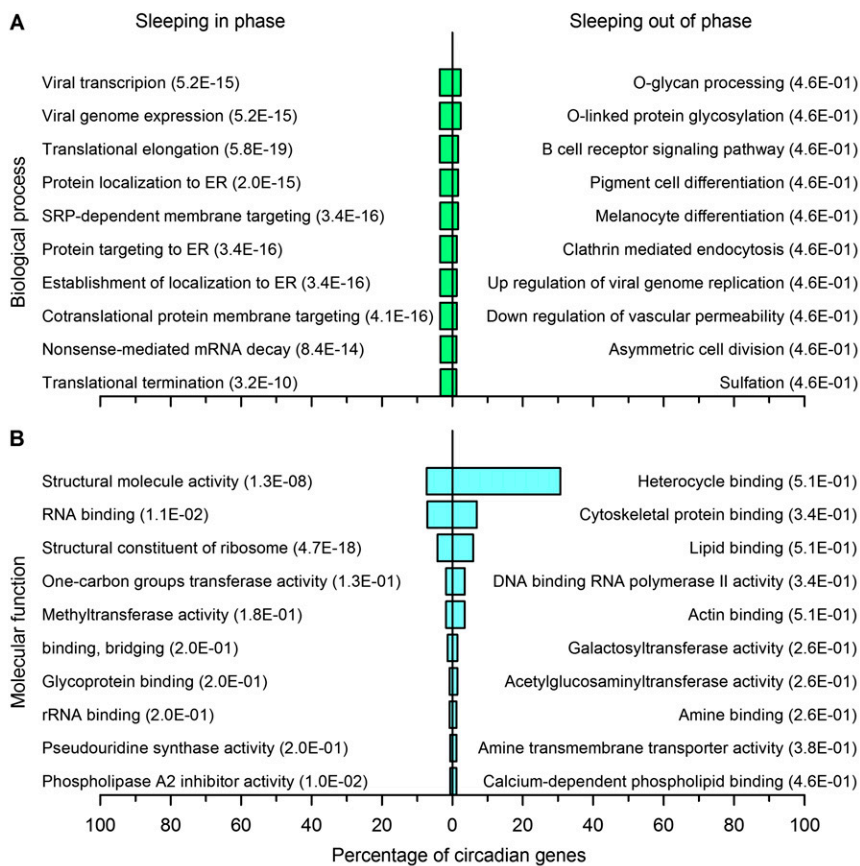

Fig. S2. Gene ontology (GO) analyses for transcripts that were classified as circadian when sleeping in phase and out of phase with melatonin. Top 10 GO biological processes (*A*) and molecular functions (*B*) associated with transcripts whose expression profiles were classified as circadian when sleeping in phase with melatonin (left side) and separately for those that were circadian when sleeping out of phase with melatonin (right side) (n = 19 paired subjects).

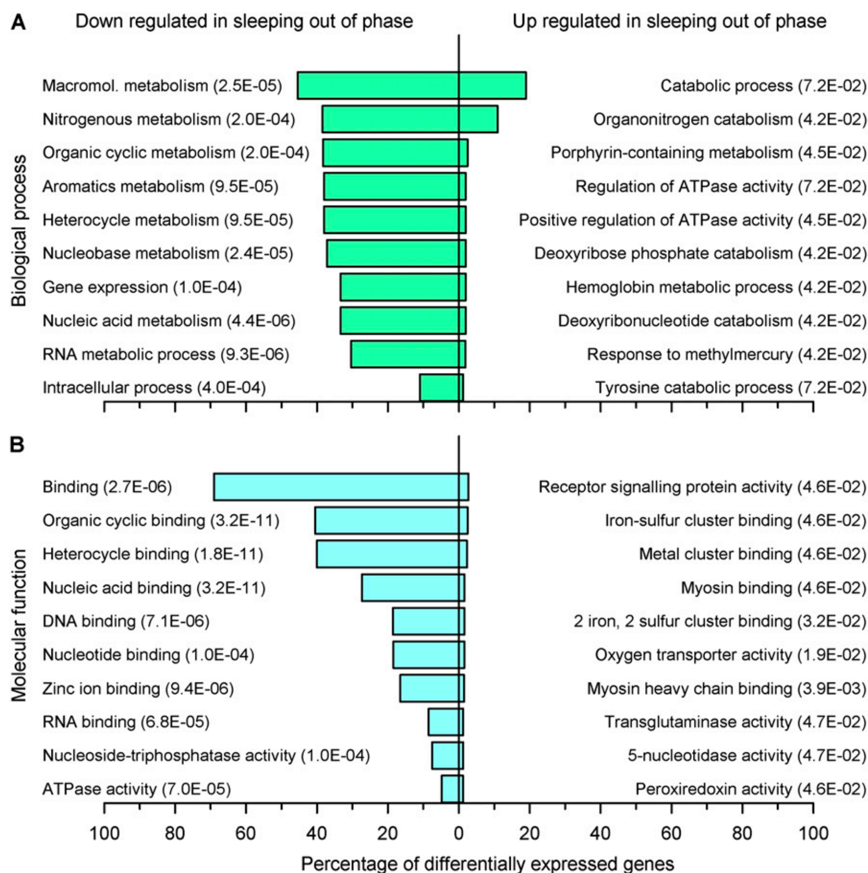

Fig. S3. GO analyses for transcripts that showed a main effect of sleep condition and were up or down-regulated. Top 10 GO biological processes (*A*) and molecular functions (*B*) associated with transcripts whose expression profiles showed a main effect of sleep condition and were significantly down-regulated when sleeping out of phase [left side; ANOVA; Benjamini–Hochberg (BH)-corrected $P < 0.05$; $n = 22$] or were significantly up-regulated when sleeping out of phase (right side; ANOVA; BH-corrected $P < 0.05$; $n = 22$), compared with the sleeping in-phase condition.

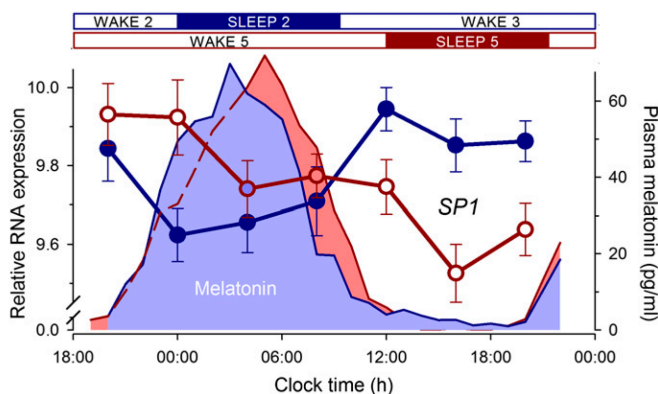

Fig. S4. Expression profiles of *SP1*. Mean expression profiles ($\log_2 \pm$ SEM) for *SP1* when sleeping in phase (blue line) and out of phase (red line) with melatonin (ANOVA interaction sleep condition × sampling time; BH-corrected $P = 0.001$; $n = 22$).

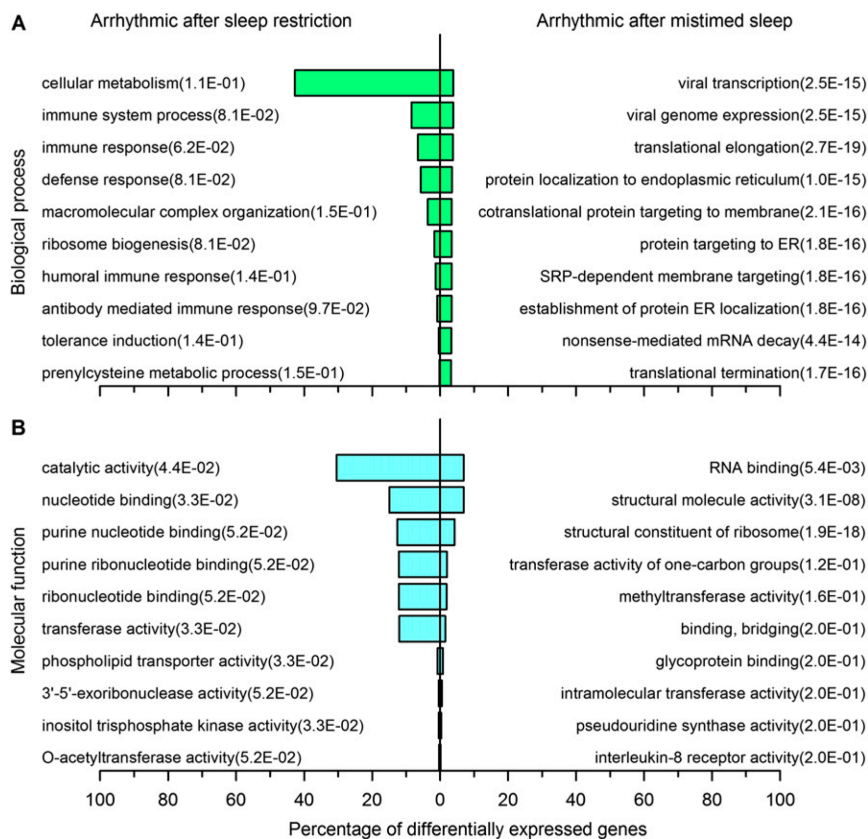

Fig. S5. GO analyses for transcripts that became arrhythmic after sleep restriction compared with those that became arrhythmic during mistimed sleep. Top 10 GO biological processes (*A*) and molecular functions (*B*) associated with transcripts whose expression profiles became arrhythmic during total sleep loss after sleep restriction in a previous study (left side) (1) and those that became arrhythmic during mistimed sleep in the present study (right side).

1. Möller-Levet CS, et al. (2013) Effects of insufficient sleep on circadian rhythmicity and expression amplitude of the human blood transcriptome. *Proc Natl Acad Sci USA* 110(12): E1132–E1141.

Fig. S6. GO analyses for transcripts that showed a main effect of sleep condition after sleep restriction or during mistimed sleep. Top 10 GO biological processes (*A*) and molecular functions (*B*) associated with transcripts whose expression profiles showed a main effect of sleep restriction vs. sufficient sleep in a previous study (left side) (1) or a main effect of mistimed sleep vs. sleeping in phase in the present study (right side).

1. Möller-Levet CS, et al. (2013) Effects of insufficient sleep on circadian rhythmicity and expression amplitude of the human blood transcriptome. *Proc Natl Acad Sci USA* 110(12): E1132–E1141.

Fig. S7. Overlap between genes whose expression showed an interaction between sleep condition and time according to ANOVA and genes targeted by CIRBP and RBM3. The Venn diagram shows the overlap between genes whose transcripts showed an interaction between sleep condition and sample time with equivalent genes (converted using MADGene) identified as being targets for CIRBP ($P = 0$) and RBM3 ($P = 1.34 \times e^{-10}$) binding in mice (2).

Table S1. Study participant demographics

Characteristic	Males	Females	Total
N	11	11	22
Age, y	25.2 (3.1)	27.3 (3.5)	26.3 (3.4)
Body mass index, kg/m^2	23.0 (1.7)	21.0 (1.8)	22.0 (2.0)
Habitual bedtime from actigraphy and sleep diary	0027 hours (58 min)	2338 hours (1 h and 5 min)	0002 hours (1 h and 5 min)
Habitual sleep duration	7 h and 37 min (52 min)	7 h and 57 min (57 min)	7 h and 47 min (52 min)

Values in parentheses are ±SD.

Table S2. GO processes for in-phase circadian transcripts that peak during the day and during the night

Biological process	*P*
Peak in day	
Response to wounding	0.002
Defense response	0.0013
Positive regulation of angiogenesis	0.0046
Negative regulation of vasoconstriction	0.005
Response to stress	0.005
Interleukin-8 binding/receptor activity	0.022
Cytokine receptor activity	0.022
Calcium-activated potassium channel activity	0.032
Peptide receptor activity	0.03
G protein coupled peptide receptor activity	0.03
Hormone activity	0.045
Peak in night	
Protein targeting to the ER	3.51×10^{-21}
SRP-dependent protein targeting to membrane	3.51×10^{-21}
Translation initiation	1.3×10^{-7}
Translation elongation	7.4×10^{-23}
Translation termination	1.51×10^{-22}
mRNA catabolic process nonsense mediated decay	3.51×10^{-21}
Structural constitute of ribosome	2.96×10^{-28}
RNA binding	5.18×10^{-13}
rRNA binding	0.0002
Methyltransferase activity	3.03×10^{-5}
Transferase activity	3.54×10^{-5}
Lymphocyte differentiation	0.038
Lymphocyte activation	0.038
T-cell differentiation	0.038
T-cell activation	0.038

Table S3. ANOVA interaction genes with biologically confirmed binding sites for SP1

Cell line	ANOVA interaction gene list enrichment, %	Mean enrichment of simulations, %	*P*
GM12878	31.77 (2,894)	20.88	$<2.2 \times e^{-16}$
HepG2	23.48 (2,139)	16.13	$<2.2 \times e^{-16}$
H1-hESC	23.25 (2,118)	15.92	$<2.2 \times e^{-16}$
K562	18.02 (1,642)	12.11	$<2.2 \times e^{-16}$

Values in parentheses refer to number of genes.

Other Supporting Information Files

Dataset S1 (XLSX)
Dataset S2 (XLSX)
Dataset S3 (XLSX)