bioRxiv preprint doi: https://doi.org/10.1101/628172; this version posted September 23, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Title:

Longitudinal transcriptome-wide gene expression analysis of sleep deprivation treatment shows involvement of circadian genes and immune pathways

Authors:

Jerome C. Foo[1], Nina Trautmann[1,2,3], Carsten Sticht[4], Jens Treutlein[1], Josef Frank[1], Fabian Streit[1], Stephanie H. Witt[1], Carolina De La Torre[4], Steffen Conrad von Heydendorff[2], Lea Sirignano[1], Junfang Chen[2], Bertram Müller-Myhsok[5,6,7], Andreas Meyer-Lindenberg[2], Christian C. Witt[8], Maria Gilles[2], Michael Deuschle[2], Marcella Rietschel[1]

Affiliations:

[1]Department of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany

[2]Department of Psychiatry and Psychotherapy, Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany

[3]Department of Child and Adolescent Psychiatry and Psychotherapy, Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany

[4]Medical Research Center, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany

[5]Department of Translational Research in Psychiatry, Max Planck Institute of Psychiatry, Munich, Germany.

[6]Munich Cluster of Systems Biology, SyNergy, Munich, Germany

[7]Institute of Translational Medicine, University of Liverpool, Liverpool, United Kingdom

bioRxiv preprint doi: https://doi.org/10.1101/628172; this version posted September 23, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

[8]Department of Anaesthesiology and Operative Intensive Care, University Hospital Mannheim, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany

Correspondence to:

Jerome Clifford Foo, PhD

Department of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, J5, 68159

Email: jerome.foo<at>zi-mannheim.de

Phone: +49-621-1703-6093; Fax: +49-621-1703-6055

Running Title:

Sleep deprivation treatment and transcriptome-wide gene expression

Keywords:

Depression; Sleep Deprivation; Gene Expression; Transcriptome; Circadian; Immune

bioRxiv preprint doi: https://doi.org/10.1101/628172; this version posted September 23, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Abstract:

Background

Therapeutic sleep deprivation (SD) rapidly induces robust, transient antidepressant effects in a large proportion of major mood disorder patients suffering from a depressive episode, but underlying biological factors remain poorly understood. Research suggests that these patients may have altered circadian molecular genetic 'clocks' and that SD functions through 'resetting' dysregulated genes; additional factors may be involved, warranting further investigation. Leveraging advances in microarray technology enabling the transcriptome-wide assessment of gene expression, this study aimed to examine gene expression changes accompanying SD and recovery sleep in patients suffering from an episode of depression.

Methods

Patients (N=78) and controls (N=15) underwent SD, with blood taken at the same time of day before, after one night of SD and after recovery sleep. A transcriptome-wide gene-by-gene approach was used, with a targeted look also taken at circadian genes. Furthermore, gene set enrichment, and longitudinal gene set analyses including the time point after recovery sleep, were conducted.

Results

Circadian genes were significantly affected by SD, with patterns suggesting that molecular clocks of responders and non-responders, as well as patients and controls respond differently to chronobiologic stimuli. Notably, gene set analyses revealed a strong widespread effect of SD on pathways involved in immune function and inflammatory response, such as those involved in cytokine and especially in interleukin signalling. Longitudinal gene set analyses showed that in responders these pathways were upregulated after SD; in non-responders, little response was observed.

Conclusions

bioRxiv preprint doi: https://doi.org/10.1101/628172; this version posted September 23, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Our findings emphasize the close relationship between circadian, immune and sleep systems and their link to etiology of depression at the transcriptomic level.

bioRxiv preprint doi: https://doi.org/10.1101/628172; this version posted September 23, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Introduction:

Therapeutic sleep deprivation (SD) rapidly induces robust antidepressant effects in a large proportion of major mood disorder patients suffering from a depressive episode (1-5) . The effects of the treatment are transient as relapse is usually observed after recovery sleep. The mechanisms through which SD exerts its antidepressant effects nevertheless offer important insights into the biological factors involved in depression and antidepressant response, and have been the focus of recent research (6-10). Work in humans (11-14)as well as animals (15-17) consistently documents the effects of mistimed or insufficient sleep and sleep deprivation on circadian gene expression (such as *CLOCK, ARNTL [BMAL1], PER1, PER2, PER3, etc.,)* as well as on genes involved in related biological processes such as inflammatory, immune and stress response (18, 19). A prominent hypothesis about the antidepressant mechanism underlying SD is that it restores circadian rhythmicity which is often dysregulated in depression, via resetting clock gene transcription (20, 21). The well-controlled nature and rapidity of response to SD treatment (22) renders it a promising context to investigate associated biological measures such as gene expression.

While no systematic investigation of gene expression changes in depressed patients undergoing SD treatment has been conducted to date, the study of gene expression in major depressive disorder (MDD) has raised the idea that genes associated with MDD are enriched for inflammation and immune response pathways, which may be linked to the sleep disturbances observed in depression (23-25). Circadian rhythms are found in the majority of physiological processes and the immune system is no different, with alterations of these rhythms leading to disturbed immune responses (26). The immune system and circadian clock circuitry crosstalk, with immune challenges and mediators, such as cytokines, also feeding back to affect circadian rhythms (27). Cytokines, including chemokines, interferons and interleukins, are integral to sleep homeostat regulation and can modulate behavioural and physiological functions (28).

bioRxiv preprint doi: https://doi.org/10.1101/628172; this version posted September 23, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

We recently conducted a naturalistic study which aimed to examine clinical and genetic factors predicting response to SD (29). This was conducted in a sample of major mood disorder inpatients experiencing a depressive episode (n=78) and healthy controls (n=15). Briefly, 72% of patients responded to SD. Responders and non-responders did not differ in self/expert assessed symptom ratings or chronotype, but mood differed. Response was associated with lower age and later age at lifetime disease onset. Higher genetic burden of depression was observed in non-responders than healthy controls, with responders having intermediate risk scores.

The present study now aimed to examine gene expression changes accompanying SD and recovery sleep during a depressive episode using a longitudinal design, looking at changes in peripheral blood gene expression in the same sample. Gene expression changes occurring after SD and recovery sleep, as well as associated with response and patient-control status, were explored. In a hypothesis-driven approach, expression patterns of circadian genes were investigated. For a systematic search, transcriptome-wide gene-by-gene and gene set enrichment analyses were performed, while longitudinal gene set analysis (30) explored dynamics in expression trajectories at the functional gene set level.

bioRxiv preprint doi: https://doi.org/10.1101/628172; this version posted September 23, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Methods and Materials:

Participants:

The present sample has been described elsewhere (29). Seventy-eight inpatients (34 females; age mean ± standard deviation = 43.54 ± 14.80 years) presenting with a depressive episode (unipolar, n = 71; bipolar I, n = 6; and bipolar II, n = 1) and on stable medication for ≥ 5 days participated in this study. Depression was diagnosed according to ICD-10 criteria. Patients were recruited from consecutive admissions to the depression unit of the Department of Psychiatry and Psychotherapy of the Central Institute of Mental Health (CIMH), Mannheim, Germany. Prescribed medication included typical and atypical antidepressants, lithium, and adjunct therapies (anticonvulsants, antipsychotics and sleeping agents). Fifteen healthy controls (8 females; 40.53 ± 15.90 years) with no history of psychiatric/somatic disorders were recruited through an online advertisement on the CIMH website. The criteria for inclusion and exclusion were the same as the criteria for patients, except controls needed to lack psychiatric diseases, which was evaluated via Structured Clinical Interview for DSM-IV Axis II disorders (SCID-II) prior to SD. The investigation was carried out in accordance with the Declaration of Helsinki and approved by the CIMH ethics committee. All participants provided written informed consent following a detailed explanation of the study.

Sleep Deprivation

On Day 1, participants gave informed consent and entered the study (see **Figure 1**). SD was conducted from Day 2–Day 3, in small groups of 1-5 participants under staff supervision. Participants were free to move around during the night of the SD protocol and were supervised by staff and occupied with activities such as games and walks to ensure wakefulness. Resting in bed was not allowed and SD was carried out on the ward under regular ward lighting conditions (no bright or dim lighting). Food intake was not restricted and some patients consumed snacks (i.e. bread) during SD. Participants underwent the same protocol irrespective of habitual sleep/wake timing; patients

bioRxiv preprint doi: https://doi.org/10.1101/628172; this version posted September 23, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

followed ward routines (i.e. lights on, lights out times) prior to inclusion to the study. Participants remained awake from ~0600hrs on Day 2 to 1800hrs on Day 3 (36 hours). On Day 3, participants underwent recovery sleep from 1800-0100hrs. Sleep phase advance was then carried out, shifting sleep one hour forward each day until the patient's regular sleep pattern was reached. Controls participated alongside patients. Response was assessed by the senior clinical researcher using the Clinical Global Impression Scale for Global Improvement/Change in the afternoon on Day 3.

Data Collection

Blood samples were collected in RNA-stabilizing PAXgene tubes (Qiagen, Hilden, Germany), processed according to standard procedures, and stored at −80°C until analysis. Blood was collected at the same time (between 0600–0730hrs) on Day 2 before SD (T1), on Day 3 after SD (T2) and on Day 4 (T3) (see **Figure 1**). The number of samples decreased over time points (**Table 1**) due to non-participation.

Sample preparation and analysis

RNA and Microarray Analyses

Laboratory analyses were performed using standard methods (see **Supplementary Information**, **SI**).

Gene Expression Data Pre-processing

A Custom CDF Version 20 with ENTREZ based gene definitions was used to annotate the Affymetrix GeneChip™ Human Gene 2.0 ST arrays used for gene expression profiling (31). The Raw fluorescence intensity values were normalized applying quantile normalization and RMA background correction

bioRxiv preprint doi: https://doi.org/10.1101/628172; this version posted September 23, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

using SAS JMP11 Genomics, version 7(SAS Institute, Cary, NC, USA). The final dataset comprised mRNA expression targeting 24,733 unique genes for each time point per participant.

Data Analysis

Analyses were conducted in R (Microsoft R Open 3.4.2). Significance was set at FDR $q < 0.05$.

Gene-based Analysis

Analyses were conducted using lme4 (Version 1.1-17). Linear mixed effects models with random intercepts were fitted to examine gene expression differences between T1 and T2 ('effect of time point'). Three main models were fitted: effect of time point in 'all patients' (M1), in 'responders vs. non-responders' (M2) and in 'patients vs. controls' (M3). In all analyses, age and sex were included as covariates.

Models were specified as follows: for each transcript, likelihood ratio tests were calculated between two models (h1 vs h0). In both models, gene expression was specified as the dependent variable, covariates were specified as fixed effects and the individual was specified as a random effect. h1 contained the comparison of interest specified as a fixed effect while h0 was a reduced model without it. That is, in M1, the 'effect of time point' was the only difference between h1 and h0, while in M2 and M3, the effect of interest was the interaction of the comparison group status (i.e. responder/non-responder, and patient/control) and 'effect of time point'.

Additionally, whether differences in expression levels at T1 'baseline' were informative about response and disease status was examined using linear models, controlling for sex and age.

Targeted Examination of Differential Expression of Circadian Genes

bioRxiv preprint doi: https://doi.org/10.1101/628172; this version posted September 23, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

To determine differential expression of circadian genes, a list of genes comprising both traditional clock genes (in order of highest signal-to-noise ratio predicting circadian rhythmicity): *PER1, NR1D2,PER3, NR1D1, PER2, ARNTL, NPAS2, CLOCK, CRY2*, and *CRY1* as well as other top genes shown to predict circadian rhythmicity in human blood (*DDIT4, CLEC4E, FKBP5, DAAM2, TPST1, IL13RA1, SMAP2, HNRNPDL, FOSL2, PER1, FLT3, CDC42EP2, TMEM88, NR1D2, RBM3*) in (32) was used to take a focused look at results of the gene-based analysis. Using a Monte-Carlo approach, the probability of obtaining at least the observed number of significant associations ($p < 0.05$ uncorrected) in a random gene set of the same size was calculated.

Gene Set Enrichment Analysis

Gene Set Enrichment Analysis (GSEA)(33) was used to determine whether differentially expressed genes offered biologically meaningful insights about SD. Ranked lists were created based on results of models M1-M3, using a signed log10 transformed p-value with sign denoting direction of change, as described elsewhere(34). To allow a concise interpretation of the potentially widespread effects of SD interventions, the Hallmark gene set collection (35) (MSigDB Version 6.2), comprising 50 gene sets representing specific well-defined biological states/processes displaying coherent expression, was used. The heme metabolism gene set was excluded due to the globin interference artefact (communication with ThermoFisher).

Longitudinal Gene Set Trajectory

Time-course Gene Set Analysis (TcGSA)(30) (Version 0.12.1) was employed to examine gene expression dynamics over all three time points. TcGSA, employing mixed models, detects gene sets in which expression changes over time, taking between-gene and individual variability into account, with higher sensitivity and better interpretability than univariate individual gene analysis (for details,

bioRxiv preprint doi: https://doi.org/10.1101/628172; this version posted September 23, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

see(30)). As above, the Hallmark gene set collection was used. Significance was set at FDR $q < 0.05$. TcGSA was employed separately for all patients, controls, responders and non-responders. Data at T3 from two patients not following the recovery sleep protocol were excluded, while 8 participants had dropped out.

bioRxiv preprint doi: https://doi.org/10.1101/628172; this version posted September 23, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Results:

Gene-based Analysis

In all patients [M1], 4,071 (2,083 up, 1,988 down) genes were significantly differentially expressed after SD. Significant differential changes in gene expression after SD between responders and non-responders [M2] were observed in 360 genes [150 up, 210 down] and in patients vs. controls [M3] in 495 genes [248 up, 247 down].

Table 2 shows top differentially expressed genes for these models. **Tables S1.1-S1.3** show the number of genes differentially expressed, upregulated, and downregulated for all models, and detailed lists of differentially expressed genes.

At baseline, no genes were significantly differentially expressed between patients/controls and responders/non-responders (**Table S1.4**).

Target Examination of Differential Expression of Circadian Genes

We observed significant differential expression of circadian genes in models M1, M2 and M3. (**Table 3**, for more details, see **Tables S2.1-3**). Baseline differences in circadian genes between responders/non-responders, and patients/controls did not reach FDR $q < 0.05$, but achieved nominal significance (see **Table S2.4**).

Monte Carlo simulations confirmed that significantly more circadian genes were differentially expressed than random gene sets of the same size (see **SI**).

Gene Set Enrichment Analysis

GSEA notably found enrichment in immune response related pathways (see **Tables S3.1-3**).

bioRxiv preprint doi: https://doi.org/10.1101/628172; this version posted September 23, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

For M1, M2 and M3, 12, 23 and 11 gene sets were significantly positively enriched, respectively (FDR $q < 0.05$). The *TNFα signalling via NFKβ* gene set had the strongest positive enrichment in all models, while *Inflammatory Response* was also consistently among the significantly positively enriched gene sets.

Given the enrichment observed in Tumor Necrosis Factor Alpha (TNFα) and immune pathways, selected genes prominent in immune processes(25) were further examined (**Table S4**).

Time course Gene Set Analysis

TcGSA results mirrored and extended gene-based analysis results:

In responders, 48 gene sets varied significantly (the model for one gene set, G2M Checkpoint, did not converge) (see **Figure 2a**). Descriptively, in comparison to T1, responders showed a spectrum of differential gene expression at T2, with strong upregulation observed (TNFα Signalling via NFKβ, IL6-JAK-STAT3-Signaling, Inflammatory Response, Angiogenesis) and maintained until T3. The Interferon Gamma Response and Interferon Alpha Response gene sets showed the strongest upregulation at T3.

In non-responders, 44 gene sets varied significantly (the model for one gene set, Allograft Rejection, did not converge)(see **Figure 2b**), the majority of gene sets were downregulated at T2, with upregulation of the abovementioned gene sets only observed at T3.

In patients, expression was observed to vary significantly in 49 gene sets (see **Figure 2c**). Descriptively, upregulation was observed in immune system related gene sets in two main patterns; (1) strong upregulation at T2, sustained but weakening at T3 (i.e. Inflammatory Response, IL6-Jak-Stat3-Signalling and TNFα Signalling via NFKβ) and (2) light upregulation at T2 followed by stronger upregulation at T3 (e.g., Interferon Gamma Response, Interferon Alpha Response).

bioRxiv preprint doi: https://doi.org/10.1101/628172; this version posted September 23, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

In controls, 21 gene sets varied significantly (**see Figure 2d**). Descriptively, at T3, immune/inflammation related gene sets were upregulated (e.g., Inflammatory Response, IL6-Jak-Stat3-Signalling, TNFα Signalling via NFKβ). The majority of other gene sets were downregulated at T2, followed by upregulation at T3.

Tables S5.1-4 list significant TcGSA gene sets.

bioRxiv preprint doi: https://doi.org/10.1101/628172; this version posted September 23, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Discussion:

Here, we report the first longitudinal transcriptome-wide study of SD treatment in major mood disorder patients suffering from a major depressive episode. Widespread differential gene expression was observed after SD; circadian genes were differentially expressed, and enrichment in pathways related to immune function, inflammatory response and sleep regulation was observed.

It has been hypothesized that SD exerts its antidepressant effect by resetting disturbed clock gene functioning thought to be a feature of depression (9). Consequently, we examined changes in expression of circadian genes. All individuals, i.e. patients and controls, showed significant differential expression of circadian genes after SD. In responders, many significant gene expression changes were observed while in non-responders only a few nominally significant changes were found. The following observation may be of interest: three circadian genes, *PER1, CLEC4E* and *SMAP2* (FDR q = 0.083 approaching significance, 0.048, and 0.039, respectively) showed strong differential gene expression, i.e. increased expression, in responders versus non-responders after SD. While the roles of *CLEC4E* and *SMAP2* are yet unexplored in this context (however, see below for additional discussion of *CLEC4E*), similar *PER1*-related findings have been previously reported; consistent with the present findings, *PER1* expression is observed to be increased in SD responders and decreased in non-responders (9). Animal studies have shown that sleep deprivation or prolonged wakefulness enhanced *Per1* expression in several brain regions (20, 36), and that quetiapine increased *Per1* expression in the amygdala (37).

The present results are interesting in light of a study in human post-mortem brain tissue of ~12,000 ranked genes according to robustness of circadian rhythmicity across 6 brain regions; the top ranked circadian genes in that study were *ARNTL*, *PER2*, *PER3* and *NR1D1* and dysregulation in MDD vs controls were observed in these genes (21). The present study observed that these same genes were the most affected (of the traditional clock genes) as a result of SD, supporting the idea that SD may act on the dysregulation of these genes in depression.

bioRxiv preprint doi: https://doi.org/10.1101/628172; this version posted September 23, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

The present findings cannot yet demonstrate that clock gene dysregulation/normalization is at the core of the SD mechanism. However, they suggest that (genes of) the molecular clocks of responders/non-responders, as well as patients/controls respond differently to chronobiologic stimuli, which may be associated with treatment outcomes.

Among the top 10 genes most significantly differentially expressed after SD in patients [M1] were: i) *KLF6*, observed to be upregulated in individuals after experimental restriction in a genome-wide association study of sleep duration (38); (ii) *SIPA1L1*, expression of which was found to be increased in a study examining changes in military personnel at baseline and after improvement of sleep (39); (iii) *NHSL2*, the function of which is unknown, but which is located in a genomic region found to be differentially hydroxymethylated in a study of sleep deprivation in mice(40); (iv) *TREM1*, which has immune function(11); and (v) *TSPAN2*, of which a study looking for gene expression changes under fluoxetine in rats found hippocampal upregulation (41). Among the top 10 genes observed in responders vs non-responders [M2] is *BECN1* (Beclin1) – which (together with *FKBP5,* see below) is shown to be involved in priming autophagic pathways to set the stage for antidepressant action (42, 43). Circadian rhythms of autophagic proteins, including beclin1 have been linked to sleep disturbances (44). Also in the top 10 in M2 were *DNER,* and *GSR* which have functions related to immune response(45, 46). Between patients and controls [M3], top genes included *EZH2,* which is reported to have a close relationship to IL-6 (47). *ZBTB16* is implicated in human sleep duration(38) and sleep deprivation in animal studies (48) , and *CISH* is shown to be involved in immune related processes (49, 50). Among other differentially expressed genes are ones evidently associated with antidepressive intervention, such as *FKBP5* (51) where we observed significant upregulation in patients vs. controls after SD (see **Tables S1.1, S2.1**). *FKBP5* plays an important regulatory role in stress response (51-54); circadian rhythm abnormalities have been linked to the stress response system, and circadian clock genes can both regulate and be regulated by rhythms of glucocorticoid release (55).

bioRxiv preprint doi: https://doi.org/10.1101/628172; this version posted September 23, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Pathway analyses showed a global effect of SD on gene expression; immunological, inflammatory response and sleep regulation involved pathways were most strongly affected. These findings are of interest in light of previous gene expression studies linking immune function to MDD (56, 57); SD may affect pathways involved in the MDD etiology. In patients and especially responders, in the *Inflammatory Response* (genes related to cytokines, growth factors, cell differentiation markers and transcription factors)(35), *IL6-JAK-STAT3-Signalling* (aberrantly hyperactivated in patients with cancer and chronic inflammatory conditions)(58) , and *TNFα Signalling via NFKβ* (cell proliferation, differentiation, apoptosis, neuroinflammation mediated cell death) gene sets, strong upregulation was observed at T2. Prior findings have shown associations between the immune system and depression, suggesting that causal pathways exist from immune dysregulation and inflammation to MDD (23, 24, 59-62). This raises the question of how SD and the immune system interact and whether SD counteracts and/or enhances depression-related immunological processes.

Interestingly, and in contrast to responders, mainly weaker downregulation was observed at T2 in non-responders, with upregulation of immune related gene sets only observed at T3. This differential function of immune related processes (i.e. blunted system responsiveness) might be associated with non-response to treatment. This preliminary finding must be further explored in larger sample sizes.

Of note, downregulation of *MYC targets* pathways after SD was consistently observed in both responders and non-responders but not controls. *MYC*, well known as an oncogene (63), acts as a mediator and coordinator of cell behaviour which also inversely modulates the impact of the cell cycle and circadian clock on gene expression (64). It has been shown that dysregulation of *MYC* disrupts the molecular clock by inducing dampened expression and oscillation of CLOCK-BMAL1 master circadian transcription factor (65). Removal of clock repression by *MYC* may play a role in the interaction of SD with depression and further investigation of these potential mechanisms is warranted.

bioRxiv preprint doi: https://doi.org/10.1101/628172; this version posted September 23, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Sleep and immunity are connected by anatomical and physiological bases (66). The role of cytokines in the brain is complex and remains to be fully understood; while a full discussion of their roles is beyond the scope of this work, **Table S4** shows cytokines and substances which have been implicated in both immune and sleep regulatory processes (25). The present findings, observing upregulation after SD, support reports linking levels of pro-inflammatory cytokines to depression and suggesting involvement in disease pathogenesis (25, 67, 68).

One important clue to SD response may lie in the observed TNFα expression patterns. TNFα is a pro-inflammatory cytokine controlling expression of inflammatory genetic networks; in addition to many immune-related functions in the brain, it influences whole organism function, including sleep regulation (25, 60). After SD, patients had significantly decreased expression of *TNF* compared to controls (FDR q = 0.01); upregulation was observed in controls and downregulation in patients. It should be noted here that the sample sizes were unbalanced, potentially introducing bias in the result, however, upregulated *TNF* in controls is consistent with reports of sleep deprivation-induced increases in TNFα levels in healthy people (69). The decrease observed in patients, and especially responders, may inform the mechanism of SD response- increased TNFα concentrations are reported to be a marker of depression and TNFα administration is reported to induce depressive symptoms (70).

Sleep-wake cycles are accompanied by changes in circulating immune cell numbers and disturbing the circadian cycle affects immune response (71, 72). Acute SD reportedly affects diurnal rhythmicity of cells, mirroring the immediate immune stress response (73, 74). Consistent with a stress-like response, upregulation of expression of *IL6* and *IL1B*, thought to be involved in depression (75) and regulation of circadian rhythm/sleep homeostasis (12, 68, 76), was observed after SD. Given the tight coupling between circadian and immune systems, it may be that like the 'resetting' of circadian genes, the altered rhythmicity of the stress immune response system in depression is transiently normalized, leading to antidepressant effects; chronotherapeutic approaches that extend SD effects may be prolonging this normalization (20).

bioRxiv preprint doi: https://doi.org/10.1101/628172; this version posted September 23, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

In support of the idea of involvement of stress response associated with SD is the fact that several of the differentially expressed genes are known to be driven by glucocorticoid signalling. One possibility is that differential expression observed has been induced by stress responses associated with SD; it is not possible in the present protocol to disentangle stress and SD. For example, while as indicated above *CLEC4E* is not explored in the SD context, it is known to be involved in immune function and also to be driven by glucocorticoids and is thus related to the stress response (77). Animal studies have shown that by removing glucocorticoid signalling via adrenalectomy, PER1, for example, was no-longer affected by SD, and responds to the stress induced by SD rather than SD itself (78).

There are several limitations to our study. The sample sizes of non-responders and controls were limited, with less power potentially contributing to the lower number of significant genes. The differences in sample sizes might furthermore create bias in the analyses of differential gene expression. While the statistical approach (linear mixed models) used in the single-gene analysis and underlying TcGSA is robust against unbalancedness and missing data, caution should still be exercised when considering the comparative results, as mentioned above. Next, although blood was sampled once per day on three consecutive days, a significant but manageable load for patients, the amount of data is sparse for statistical estimation. To better leverage longitudinal data and methods, a denser sampling scheme (e.g. every few hours) will be required to attain a more refined understanding of underlying SD mechanisms. It should be noted that the recovery sleep episode was a total of 7 hrs in length (i.e. 1800 to 0100 hrs) after a 36 hr homeostatic buildup of sleep pressure; this may not be long enough for homeostasis to recover and thus SD could have still had an effect on gene expression at T3. To address this in more detail, however, a different experimental design would have been required. Also, while food intake was not given special emphasis in this study, food intake can be an entrainment signal for the circadian clock. In the present study, some participants had small or simple meals (i.e. bread) and food intake was not restricted; possible effects cannot be excluded in the present design. Another direction which would be informative about circadian phase would be to include a marker of the central clock phase, e.g. melatonin secretion, also sampled at

bioRxiv preprint doi: https://doi.org/10.1101/628172; this version posted September 23, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

high temporal resolution. The sample studied was a naturalistic sample recruited from consecutive admissions and treated following standardized clinical guidelines. Medication regimens were tailored to the individual based on specific need, resulting in a variety of therapies used. While the effects of particular medications were not examined, to control for effects on results of the study, as an inclusion criterion, it was stipulated that the patient had to be on stable medication for at least 5 days prior to SD. Given the variety of medications used it was not possible to test for associations with drug response with sufficient statistical power. However, there was no apparent difference in substance class across response status. Nevertheless, robust effects of SD were observed, perhaps in part attributable to the consistent methodology for applying SD treatment in a relatively large cohort for SD. Considering the fact that depression is a heterogeneous phenotype accompanied by a heterogeneous immunophenotype (60), means that even larger sample sizes will be needed to substantiate the present findings. Finally, we investigated peripheral tissue transcriptome-wide expression changes; although an easily obtained, valuable proxy, the correlation of expression in the blood with expression in the brain, where depression is thought to act, is imperfect and requires further study (79). On the other hand, it is precisely these cells that best represent the current status of the immune system and the inflammatory response, the gene expression of which appears at the centre of SD. In addition, the crosstalk of the organs and biorhythm tuning is also mediated via the blood system.

Our findings affirm and emphasize the close relationship between circadian, immune and sleep systems in depression at the transcriptomic level, but the directionality of cause-effect remains unclear. Circadian, immune and sleep dysregulation may precede, accompany or come as a result of depression; they now represent targets for treatment which have the ability to influence clinical outcomes. Closer investigation of these systems with larger sample sizes and denser, longer-term sampling schemes will be key to disentangling and understanding the multi-level interactions occurring.

bioRxiv preprint doi: https://doi.org/10.1101/628172; this version posted September 23, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Acknowledgements:

This work has been supported by the Deutsche Forschungsgemeinschaft (DFG), SCHW 1768/1-1. AM-L acknowledges support from the German Federal Ministry of Education and Research (BMBF, grants 01ZX1314G, 01GS08147, 01EF1803A, 255156212 CRC 1158 subproject B09) and the Ministry of Science, Research and the Arts of the State of Baden-Wuerttemberg, Germany (MWK, grants 42-04HV.MED(16)/16/1, 42-04HV.MED(16)/27/1). MR was supported by the BMBF through grants within the e:Med research program 01ZX1314G. This work was also supported by BMBF/DLR grant 01EW1904 and BMBF / PTJ grant 031L0190A . This manuscript was uploaded to the preprint server bioRχiv.

Disclosures

AM-L has received consultant fees from Boehringer Ingelheim, Brainsway, Elsevier, Lundbeck Int. Neuroscience Foundation and Science Advances. All other authors report no biomedical financial interests or potential conflicts of interest.

bioRxiv preprint doi: https://doi.org/10.1101/628172; this version posted September 23, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

References:

1. Gillin JC. The sleep therapies of depression. Prog Neuropsychopharmacol Biol Psychiatry. 1983;7(2-3):351-64.
2. Wu JC, Bunney WE. The biological basis of an antidepressant response to sleep deprivation and relapse: review and hypothesis. Am J Psychiatry. 1990;147(1):14-21.
3. Benedetti F, Barbini B, Colombo C, Smeraldi E. Chronotherapeutics in a psychiatric ward. Sleep Med Rev. 2007;11(6):509-22.
4. Benedetti F, Colombo C. Sleep deprivation in mood disorders. Neuropsychobiology. 2011;64(3):141-51.
5. Boland EM, Rao H, Dinges DF, Smith RV, Goel N, Detre JA, et al. Meta-Analysis of the Antidepressant Effects of Acute Sleep Deprivation. J Clin Psychiatry. 2017.
6. Wolf E, Kuhn M, Normann C, Mainberger F, Maier JG, Maywald S, et al. Synaptic plasticity model of therapeutic sleep deprivation in major depression. Sleep Med Rev. 2016;30:53-62.
7. Borbely AA, Wirz-Justice A. Sleep, sleep deprivation and depression. A hypothesis derived from a model of sleep regulation. Hum neurobiol. 1982;1(3):205-10.
8. Borbély AA, Daan S, Wirz-Justice A, Deboer T. The two-process model of sleep regulation: a reappraisal. J Sleep Res. 2016;25(2):131-43.
9. Bunney BG, Bunney WE. Mechanisms of rapid antidepressant effects of sleep deprivation therapy: clock genes and circadian rhythms. Biol Psychiatry. 2013;73(12):1164-71.
10. Lavebratt C, Sjoholm LK, Soronen P, Paunio T, Vawter MP, Bunney WE, et al. CRY2 is associated with depression. PLoS One. 2010;5(2):e9407.
11. Archer SN, Laing EE, Möller-Levet CS, van der Veen DR, Bucca G, Lazar AS, et al. Mistimed sleep disrupts circadian regulation of the human transcriptome. Proc Natl Acad Sci USA. 2014;111(6):E682-91.
12. Moller-Levet CS, Archer SN, Bucca G, Laing EE, Slak A, Kabiljo R, et al. Effects of insufficient sleep on circadian rhythmicity and expression amplitude of the human blood transcriptome. Proc Natl Acad Sci U S A. 2013;110(12):E1132-41.
13. Thimgan MS, Gottschalk L, Toedebusch C, McLeland J, Rechtschaffen A, Gilliland-Roberts M, et al. Cross-translational studies in human and Drosophila identify markers of sleep loss. PLoS One. 2013;8(4):e61016.
14. Lech K, Ackermann K, Revell VL, Lao O, Skene DJ, Kayser M. Dissecting Daily and Circadian Expression Rhythms of Clock-Controlled Genes in Human Blood. J Biol Rhythms. 2016;31(1):68-81.
15. Wisor JP, O'Hara BF, Terao A, Selby CP, Kilduff TS, Sancar A, et al. A role for cryptochromes in sleep regulation. BMC Neurosci. 2002;3:20.
16. Mongrain V, La Spada F, Curie T, Franken P. Sleep loss reduces the DNA-binding of BMAL1, CLOCK, and NPAS2 to specific clock genes in the mouse cerebral cortex. PLoS One. 2011;6(10):e26622.
17. Laposky A, Easton A, Dugovic C, Walisser J, Bradfield C, Turek F. Deletion of the mammalian circadian clock gene BMAL1/Mop3 alters baseline sleep architecture and the response to sleep deprivation. Sleep. 2005;28(4):395-409.
18. Pellegrino R, Sunaga DY, Guindalini C, Martins RC, Mazzotti DR, Wei Z, et al. Whole blood genome-wide gene expression profile in males after prolonged wakefulness and sleep recovery. Physiol Genomics. 2012;44(21):1003-12.
19. Watson NF, Buchwald D, Delrow JJ, Altemeier WA, Vitiello MV, Pack AI, et al. Transcriptional Signatures of Sleep Duration Discordance in Monozygotic Twins. Sleep. 2017;40(1).
20. Bunney BG, Li JZ, Walsh DM, Stein R, Vawter MP, Cartagena P, et al. Circadian dysregulation of clock genes: clues to rapid treatments in major depressive disorder. Mol Psychiatry. 2015;20(1):48-55.
21. Li JZ, Bunney BG, Meng F, Hagenauer MH, Walsh DM, Vawter MP, et al. Circadian patterns of gene expression in the human brain and disruption in major depressive disorder. Proc Natl Acad Sci U S A. 2013;110(24):9950-5.

bioRxiv preprint doi: https://doi.org/10.1101/628172; this version posted September 23, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

22. Dallaspezia S, Benedetti F. Sleep deprivation therapy for depression. Curr Top Behav Neurosci. 2015;25:483-502.
23. Jansen R, Penninx BW, Madar V, Xia K, Milaneschi Y, Hottenga JJ, et al. Gene expression in major depressive disorder. Mol Psychiatry. 2016;21(3):339-47.
24. Mostafavi S, Battle A, Zhu X, Potash JB, Weissman MM, Shi J, et al. Type I interferon signaling genes in recurrent major depression: increased expression detected by whole-blood RNA sequencing. Mol Psychiatry. 2014;19(12):1267-74.
25. Rockstrom MD, Chen L, Taishi P, Nguyen JT, Gibbons CM, Veasey SC, et al. Tumor necrosis factor alpha in sleep regulation. Sleep Med Rev. 2018;40:69-78.
26. Labrecque N, Cermakian N. Circadian Clocks in the Immune System. J Biol Rhythms. 2015;30(4):277-90.
27. Cermakian N, Lange T, Golombek D, Sarkar D, Nakao A, Shibata S, et al. Crosstalk between the circadian clock circuitry and the immune system. Chronobiol Int. 2013;30(7):870-88.
28. Zielinski MR, Krueger JM. Sleep and innate immunity. Frontiers in bioscience. 2011;3:632-42.
29. Trautmann N, Foo JC, Frank J, Witt SH, Streit F, Treutlein J, et al. Response to therapeutic sleep deprivation: a naturalistic study of clinical and genetic factors and post-treatment depressive symptom trajectory. Neuropsychopharmacology. 2018.
30. Hejblum BP, Skinner J, Thiebaut R. Time-Course Gene Set Analysis for Longitudinal Gene Expression Data. PLoS computational biology. 2015;11(6):e1004310.
31. Dai JL. Serial analyses of gene expression (SAGE). Methods Mol Med. 2005;103:161-74.
32. Hughey JJ. Machine learning identifies a compact gene set for monitoring the circadian clock in human blood. Genome Med. 2017;9(1):19.
33. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A. 2005;102(43):15545-50.
34. Plaisier SB, Taschereau R, Wong JA, Graeber TG. Rank-rank hypergeometric overlap: identification of statistically significant overlap between gene-expression signatures. Nucleic Acids Res. 2010;38(17):e169.
35. Liberzon A, Birger C, Thorvaldsdottir H, Ghandi M, Mesirov JP, Tamayo P. The Molecular Signatures Database (MSigDB) hallmark gene set collection. Cell systems. 2015;1(6):417-25.
36. Zhang B, Gao Y, Li Y, Yang J, Zhao H. Sleep Deprivation Influences Circadian Gene Expression in the Lateral Habenula. Behav Neurol. 2016;2016:7919534.
37. Moriya S, Tahara Y, Sasaki H, Hamaguchi Y, Kuriki D, Ishikawa R, et al. Effect of quetiapine on Per1, Per2, and Bmal1 clock gene expression in the mouse amygdala and hippocampus. J Pharm Sci. 2014;125(3):329-32.
38. Ollila HM, Kettunen J, Pietilainen O, Aho V, Silander K, Kronholm E, et al. Genome-wide association study of sleep duration in the Finnish population. J Sleep Res. 2014;23(6):609-18.
39. Livingston WS, Rusch HL, Nersesian PV, Baxter T, Mysliwiec V, Gill JM. Improved Sleep in Military Personnel is Associated with Changes in the Expression of Inflammatory Genes and Improvement in Depression Symptoms. Front Psychiatry. 2015;6:59.
40. Massart R, Freyburger M, Suderman M, Paquet J, El Helou J, Belanger-Nelson E, et al. The genome-wide landscape of DNA methylation and hydroxymethylation in response to sleep deprivation impacts on synaptic plasticity genes. Transl Psychiatry. 2014;4:e347.
41. Kroeze Y, Peeters D, Boulle F, Pawluski JL, van den Hove DL, van Bokhoven H, et al. Long-term consequences of chronic fluoxetine exposure on the expression of myelination-related genes in the rat hippocampus. Transl Psychiatry. 2015;5:e642.
42. Gassen NC, Hartmann J, Schmidt MV, Rein T. FKBP5/FKBP51 enhances autophagy to synergize with antidepressant action. Autophagy. 2015;11(3):578-80.
43. Wirawan E, Lippens S, Vanden Berghe T, Romagnoli A, Fimia GM, Piacentini M, et al. Beclin1: a role in membrane dynamics and beyond. Autophagy. 2012;8(1):6-17.
44. Post RM, Kotin J, Goodwin FK. Effects of sleep deprivation on mood and central amine metabolism in depressed patients. Arch Gen Psychiatry. 1976;33(5):627-32.

bioRxiv preprint doi: https://doi.org/10.1101/628172; this version posted September 23, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

45. Greene M, Lai Y, Baella N, Dalmau J, Lancaster E. Antibodies to Delta/notch-like epidermal growth factor-related receptor in patients with anti-Tr, paraneoplastic cerebellar degeneration, and Hodgkin lymphoma. JAMA neurology. 2014;71(8):1003-8.
46. Yan J, Meng X, Wancket LM, Lintner K, Nelin LD, Chen B, et al. Glutathione reductase facilitates host defense by sustaining phagocytic oxidative burst and promoting the development of neutrophil extracellular traps. J Immunol. 2012;188(5):2316-27.
47. Murphy TM, O'Donovan A, Mullins N, O'Farrelly C, McCann A, Malone K. Anxiety is associated with higher levels of global DNA methylation and altered expression of epigenetic and interleukin-6 genes. Psychiatr Genet. 2015;25(2):71-8.
48. Wang H, Liu Y, Briesemann M, Yan J. Computational analysis of gene regulation in animal sleep deprivation. Physiol Genomics. 2010;42(3):427-36.
49. Ito S, Bollard CM, Carlsten M, Melenhorst JJ, Biancotto A, Wang E, et al. Ultra-low dose interleukin-2 promotes immune-modulating function of regulatory T cells and natural killer cells in healthy volunteers. Mol Ther. 2014;22(7):1388-95.
50. Cao G, Wang Q, Li G, Meng Z, Liu H, Tong J, et al. mTOR inhibition potentiates cytotoxicity of Vgamma4 gammadelta T cells via up-regulating NKG2D and TNF-alpha. J Leukoc Biol. 2016;100(5):1181-9.
51. Binder EB, Salyakina D, Lichtner P, Wochnik GM, Ising M, Putz B, et al. Polymorphisms in FKBP5 are associated with increased recurrence of depressive episodes and rapid response to antidepressant treatment. Nat Genet. 2004;36(12):1319-25.
52. Binder EB. The role of FKBP5, a co-chaperone of the glucocorticoid receptor in the pathogenesis and therapy of affective and anxiety disorders. Psychoneuroendocrinology. 2009;34 Suppl 1:S186-95.
53. Lekman M, Laje G, Charney D, Rush AJ, Wilson AF, Sorant AJ, et al. The FKBP5-gene in depression and treatment response--an association study in the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) Cohort. Biol Psychiatry. 2008;63(12):1103-10.
54. Menke A, Binder EB. Epigenetic alterations in depression and antidepressant treatment. Dialogues Clin Neurosci. 2014;16(3):395-404.
55. Landgraf D, McCarthy MJ, Welsh DK. Circadian clock and stress interactions in the molecular biology of psychiatric disorders. Curr Psychiatry Rep. 2014;16(10):483.
56. Blume J, Douglas SD, Evans DL. Immune suppression and immune activation in depression. Brain Behav Immun. 2011;25(2):221-9.
57. Leday GGR, Vertes PE, Richardson S, Greene JR, Regan T, Khan S, et al. Replicable and Coupled Changes in Innate and Adaptive Immune Gene Expression in Two Case-Control Studies of Blood Microarrays in Major Depressive Disorder. Biol Psychiatry. 2018;83(1):70-80.
58. Johnson DE, O'Keefe RA, Grandis JR. Targeting the IL-6/JAK/STAT3 signalling axis in cancer. Nat Rev Clin Oncol. 2018;15(4):234-48.
59. Liu Y, Ho RC, Mak A. Interleukin (IL)-6, tumour necrosis factor alpha (TNF-alpha) and soluble interleukin-2 receptors (sIL-2R) are elevated in patients with major depressive disorder: a meta-analysis and meta-regression. J Affect Disord. 2012;139(3):230-9.
60. Barnes J, Mondelli V, Pariante CM. Genetic Contributions of Inflammation to Depression. Neuropsychopharmacology. 2017;42(1):81-98.
61. Vogelzangs N, Duivis HE, Beekman AT, Kluft C, Neuteboom J, Hoogendijk W, et al. Association of depressive disorders, depression characteristics and antidepressant medication with inflammation. Transl Psychiatry. 2012;2:e79.
62. Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, et al. A meta-analysis of cytokines in major depression. Biol Psychiatry. 2010;67(5):446-57.
63. Conacci-Sorrell M, McFerrin L, Eisenman RN. An overview of MYC and its interactome. Cold Spring Harb Perspect Med. 2014;4(1):a014357.
64. Shostak A, Ruppert B, Ha N, Bruns P, Toprak UH, Project IM-S, et al. MYC/MIZ1-dependent gene repression inversely coordinates the circadian clock with cell cycle and proliferation. Nat Commun. 2016;7:11807.

bioRxiv preprint doi: https://doi.org/10.1101/628172; this version posted September 23, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

65. Altman BJ, Hsieh AL, Sengupta A, Krishnanaiah SY, Stine ZE, Walton ZE, et al. MYC Disrupts the Circadian Clock and Metabolism in Cancer Cells. Cell metabolism. 2015;22(6):1009-19.
66. Almeida CM, Malheiro A. Sleep, immunity and shift workers: A review. Sleep Science. 2016;9(3):164-8.
67. Krueger JM, Fang J, Taishi P, Chen Z, Kushikata T, Gardi J. Sleep. A physiologic role for IL-1 beta and TNF-alpha. Ann N Y Acad Sci. 1998;856:148-59.
68. Tsai SJ. Effects of interleukin-1beta polymorphisms on brain function and behavior in healthy and psychiatric disease conditions. Cytokine Growth Factor Rev. 2017;37:89-97.
69. Chennaoui M, Sauvet F, Drogou C, Van Beers P, Langrume C, Guillard M, et al. Effect of one night of sleep loss on changes in tumor necrosis factor alpha (TNF-alpha) levels in healthy men. Cytokine. 2011;56(2):318-24.
70. Pollak Y, Yirmiya R. Cytokine-induced changes in mood and behaviour: implications for 'depression due to a general medical condition', immunotherapy and antidepressive treatment. Int J Neuropsychoparmacol.. 2002;5(4):389-99.
71. Kervezee L, Cuesta M, Cermakian N, Boivin DB. Simulated night shift work induces circadian misalignment of the human peripheral blood mononuclear cell transcriptome. Proc Natl Acad Sci U S A. 2018;115(21):5540-5.
72. Aho V, Ollila HM, Rantanen V, Kronholm E, Surakka I, van Leeuwen WM, et al. Partial sleep restriction activates immune response-related gene expression pathways: experimental and epidemiological studies in humans. PLoS One. 2013;8(10):e77184.
73. Ackermann K, Plomp R, Lao O, Middleton B, Revell VL, Skene DJ, et al. Effect of sleep deprivation on rhythms of clock gene expression and melatonin in humans. Chronobiol Int. 2013;30(7):901-9.
74. Ackermann K, Revell VL, Lao O, Rombouts EJ, Skene DJ, Kayser M. Diurnal rhythms in blood cell populations and the effect of acute sleep deprivation in healthy young men. Sleep. 2012;35(7):933-40.
75. Yoshimura R, Hori H, Ikenouchi-Sugita A, Umene-Nakano W, Katsuki A, Atake K, et al. Plasma levels of interleukin-6 and selective serotonin reuptake inhibitor response in patients with major depressive disorder. Hum Psychopharmacol. 2013;28(5):466-70.
76. Cavadini G, Petrzilka S, Kohler P, Jud C, Tobler I, Birchler T, et al. TNF-alpha suppresses the expression of clock genes by interfering with E-box-mediated transcription. Proc Natl Acad Sci U S A. 2007;104(31):12843-8.
77. Laing EE, Moller-Levet CS, Poh N, Santhi N, Archer SN, Dijk DJ. Blood transcriptome based biomarkers for human circadian phase. eLife. 2017;6.
78. Mongrain V, Hernandez SA, Pradervand S, Dorsaz S, Curie T, Hagiwara G, et al. Separating the contribution of glucocorticoids and wakefulness to the molecular and electrophysiological correlates of sleep homeostasis. Sleep. 2010;33(9):1147-57.
79. Sullivan PF, Fan C, Perou CM. Evaluating the comparability of gene expression in blood and brain. Am J Med Genet B Neuropsychiatr Genet. 2006;141B(3):261-8.

bioRxiv preprint doi: https://doi.org/10.1101/628172; this version posted September 23, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Figure Legends:

Figure 1. Experimental Procedure. Patients entered the study on Day 1 and underwent sleep deprivation for ~ 36 hours from Day 2 to Day 3 before undergoing recovery sleep. Response was assessed with the CGI-C in the afternoon of Day 3 before recovery sleep. Blood for gene expression was taken at the same time (0600-0730hrs) on Days 2, 3 and 4 (T1, T2, T3 respectively).

Figure 2. Heatmaps of estimated dynamics from significant gene sets in A) responders, B) non-responders, C) patients, and D) controls. The median gene expression over subjects is used for each trend. Each trend is zeroed at T1 to represent baseline expression. Each row is a group of genes having the same trend inside a gene set, while the columns are time points. Trends are hierarchically clustered. Trends become red as median expression is upregulated or blue as it is downregulated compared to the baseline value at T1. The colour key represents the median of the standardized estimation of gene expression over the group of participants for a given trend in a significant gene set. Non-converging gene sets were excluded. For non-responders, the gene set for peroxisome was excluded from visualisation due to non-homogeneous expression within the set.

Figure 1

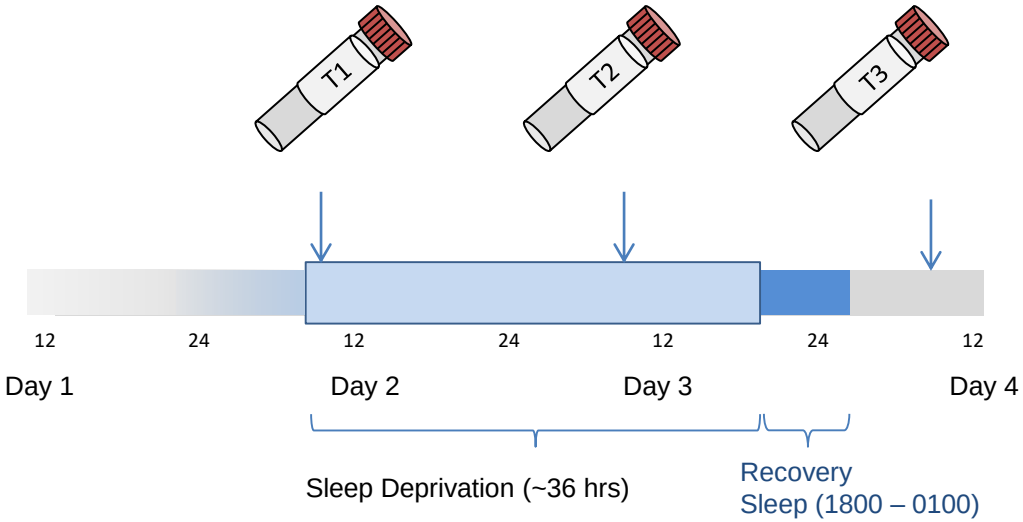
T1
T2
T3
12
24
12
24
12
24
12
Day 1
Day 2
Day 3
Day 4
Sleep Deprivation (~36 hrs)
Recovery
Sleep (1800 – 0100)

Figure 2

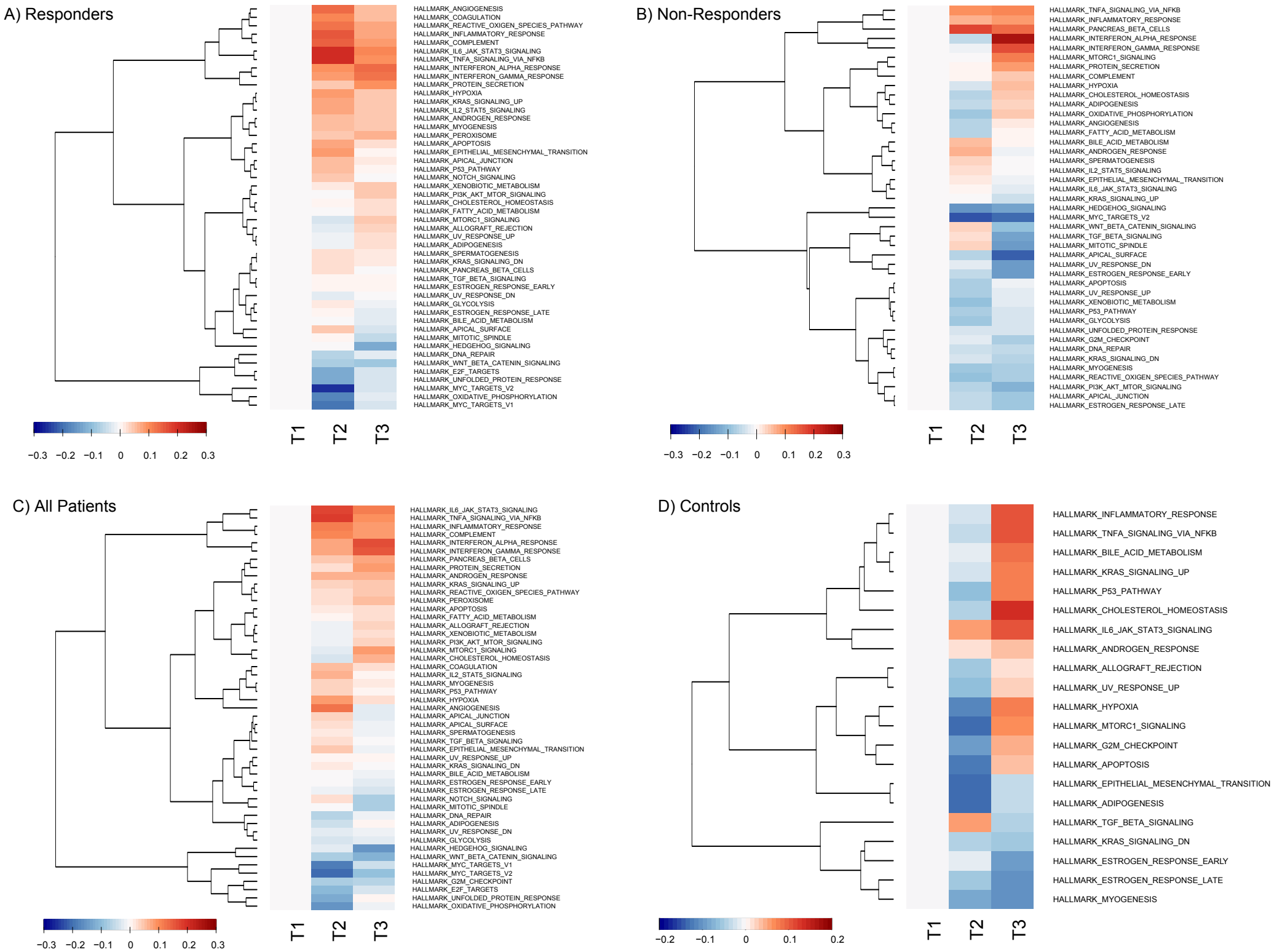
A) Responders
HALLMARK_ANGIOGENESIS
HALLMARK_COAGULATION
HALLMARK_REACTIVE_OXIGEN_SPECIES_PATHWAY
HALLMARK_INFLAMMATORY_RESPONSE
HALLMARK_COMPLEMENT
HALLMARK_IL6_JAK_STAT3_SIGNALING
HALLMARK_TNFA_SIGNALING_VIA_NFKB
HALLMARK_INTERFERON_ALPHA_RESPONSE
HALLMARK_INTERFERON_GAMMA_RESPONSE
HALLMARK_PROTEIN_SECRETION
HALLMARK_HYPOXIA
HALLMARK_KRAS_SIGNALING_UP
HALLMARK_IL2_STAT5_SIGNALING
HALLMARK_ANDROGEN_RESPONSE
HALLMARK_MYOGENESIS
HALLMARK_PEROXISOME
HALLMARK_APOPTOSIS
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION
HALLMARK_APICAL_JUNCTION
HALLMARK_P53_PATHWAY
HALLMARK_NOTCH_SIGNALING
HALLMARK_XENOBIOTIC_METABOLISM
HALLMARK_PI3K_AKT_MTOR_SIGNALING
HALLMARK_CHOLESTEROL_HOMEOSTASIS
HALLMARK_FATTY_ACID_METABOLISM
HALLMARK_MTORC1_SIGNALING
HALLMARK_ALLOGRAFT_REJECTION
HALLMARK_UV_RESPONSE_UP
HALLMARK_ADIPOGENESIS
HALLMARK_SPERMATOGENESIS
HALLMARK_KRAS_SIGNALING_DN
HALLMARK_PANCREAS_BETA_CELLS
HALLMARK_TGF_BETA_SIGNALING
HALLMARK_ESTROGEN_RESPONSE_EARLY
HALLMARK_UV_RESPONSE_DN
HALLMARK_GLYCOLYSIS
HALLMARK_ESTROGEN_RESPONSE_LATE
HALLMARK_BILE_ACID_METABOLISM
HALLMARK_APICAL_SURFACE
HALLMARK_MITOTIC_SPINDLE
HALLMARK_HEDGEHOG_SIGNALING
HALLMARK_DNA_REPAIR
HALLMARK_WNT_BETA_CATENIN_SIGNALING
HALLMARK_E2F_TARGETS
HALLMARK_UNFOLDED_PROTEIN_RESPONSE
HALLMARK_MYC_TARGETS_V2
HALLMARK_OXIDATIVE_PHOSPHORYLATION
HALLMARK_MYC_TARGETS_V1
−0.3 −0.2 −0.1 0 0.1 0.2 0.3
T1 T2 T3
B) Non-Responders
HALLMARK_TNFA_SIGNALING_VIA_NFKB
HALLMARK_INFLAMMATORY_RESPONSE
HALLMARK_PANCREAS_BETA_CELLS
HALLMARK_INTERFERON_ALPHA_RESPONSE
HALLMARK_INTERFERON_GAMMA_RESPONSE
HALLMARK_MTORC1_SIGNALING
HALLMARK_PROTEIN_SECRETION
HALLMARK_COMPLEMENT
HALLMARK_HYPOXIA
HALLMARK_CHOLESTEROL_HOMEOSTASIS
HALLMARK_ADIPOGENESIS
HALLMARK_OXIDATIVE_PHOSPHORYLATION
HALLMARK_ANGIOGENESIS
HALLMARK_FATTY_ACID_METABOLISM
HALLMARK_BILE_ACID_METABOLISM
HALLMARK_ANDROGEN_RESPONSE
HALLMARK_SPERMATOGENESIS
HALLMARK_IL2_STAT5_SIGNALING
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION
HALLMARK_IL6_JAK_STAT3_SIGNALING
HALLMARK_KRAS_SIGNALING_UP
HALLMARK_HEDGEHOG_SIGNALING
HALLMARK_MYC_TARGETS_V2
HALLMARK_WNT_BETA_CATENIN_SIGNALING
HALLMARK_TGF_BETA_SIGNALING
HALLMARK_MITOTIC_SPINDLE
HALLMARK_APICAL_SURFACE
HALLMARK_UV_RESPONSE_DN
HALLMARK_ESTROGEN_RESPONSE_EARLY
HALLMARK_APOPTOSIS
HALLMARK_UV_RESPONSE_UP
HALLMARK_XENOBIOTIC_METABOLISM
HALLMARK_P53_PATHWAY
HALLMARK_GLYCOLYSIS
HALLMARK_UNFOLDED_PROTEIN_RESPONSE
HALLMARK_G2M_CHECKPOINT
HALLMARK_DNA_REPAIR
HALLMARK_KRAS_SIGNALING_DN
HALLMARK_MYOGENESIS
HALLMARK_REACTIVE_OXIGEN_SPECIES_PATHWAY
HALLMARK_PI3K_AKT_MTOR_SIGNALING
HALLMARK_APICAL_JUNCTION
HALLMARK_ESTROGEN_RESPONSE_LATE
−0.3 −0.2 −0.1 0 0.1 0.2 0.3
T1 T2 T3
C) All Patients
HALLMARK_IL6_JAK_STAT3_SIGNALING
HALLMARK_TNFA_SIGNALING_VIA_NFKB
HALLMARK_INFLAMMATORY_RESPONSE
HALLMARK_COMPLEMENT
HALLMARK_INTERFERON_ALPHA_RESPONSE
HALLMARK_INTERFERON_GAMMA_RESPONSE
HALLMARK_PANCREAS_BETA_CELLS
HALLMARK_PROTEIN_SECRETION
HALLMARK_ANDROGEN_RESPONSE
HALLMARK_KRAS_SIGNALING_UP
HALLMARK_REACTIVE_OXIGEN_SPECIES_PATHWAY
HALLMARK_PEROXISOME
HALLMARK_APOPTOSIS
HALLMARK_FATTY_ACID_METABOLISM
HALLMARK_ALLOGRAFT_REJECTION
HALLMARK_XENOBIOTIC_METABOLISM
HALLMARK_PI3K_AKT_MTOR_SIGNALING
HALLMARK_MTORC1_SIGNALING
HALLMARK_CHOLESTEROL_HOMEOSTASIS
HALLMARK_COAGULATION
HALLMARK_IL2_STAT5_SIGNALING
HALLMARK_MYOGENESIS
HALLMARK_P53_PATHWAY
HALLMARK_HYPOXIA
HALLMARK_ANGIOGENESIS
HALLMARK_APICAL_JUNCTION
HALLMARK_APICAL_SURFACE
HALLMARK_SPERMATOGENESIS
HALLMARK_TGF_BETA_SIGNALING
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION
HALLMARK_UV_RESPONSE_UP
HALLMARK_KRAS_SIGNALING_DN
HALLMARK_BILE_ACID_METABOLISM
HALLMARK_ESTROGEN_RESPONSE_EARLY
HALLMARK_ESTROGEN_RESPONSE_LATE
HALLMARK_NOTCH_SIGNALING
HALLMARK_MITOTIC_SPINDLE
HALLMARK_DNA_REPAIR
HALLMARK_ADIPOGENESIS
HALLMARK_UV_RESPONSE_DN
HALLMARK_GLYCOLYSIS
HALLMARK_HEDGEHOG_SIGNALING
HALLMARK_WNT_BETA_CATENIN_SIGNALING
HALLMARK_MYC_TARGETS_V1
HALLMARK_MYC_TARGETS_V2
HALLMARK_G2M_CHECKPOINT
HALLMARK_E2F_TARGETS
HALLMARK_UNFOLDED_PROTEIN_RESPONSE
HALLMARK_OXIDATIVE_PHOSPHORYLATION
−0.3 −0.2 −0.1 0 0.1 0.2 0.3
T1 T2 T3
D) Controls
HALLMARK_INFLAMMATORY_RESPONSE
HALLMARK_TNFA_SIGNALING_VIA_NFKB
HALLMARK_BILE_ACID_METABOLISM
HALLMARK_KRAS_SIGNALING_UP
HALLMARK_P53_PATHWAY
HALLMARK_CHOLESTEROL_HOMEOSTASIS
HALLMARK_IL6_JAK_STAT3_SIGNALING
HALLMARK_ANDROGEN_RESPONSE
HALLMARK_ALLOGRAFT_REJECTION
HALLMARK_UV_RESPONSE_UP
HALLMARK_HYPOXIA
HALLMARK_MTORC1_SIGNALING
HALLMARK_G2M_CHECKPOINT
HALLMARK_APOPTOSIS
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION
HALLMARK_ADIPOGENESIS
HALLMARK_TGF_BETA_SIGNALING
HALLMARK_KRAS_SIGNALING_DN
HALLMARK_ESTROGEN_RESPONSE_EARLY
HALLMARK_ESTROGEN_RESPONSE_LATE
HALLMARK_MYOGENESIS
−0.2 −0.1 0 0.1 0.2
T1 T2 T3

Table 1. Samples included for analysis at different time points.

Time Point	All Subjects	Patients	Responders	Non-Responders	Controls
T1	91	76 (43M/33F)	60 (36M/24F)	16 (7M/9F)	15 (7M/8F)
T2	87	72 (41M/31F)	56 (34M/22F)	16 (7M/9F)	15 (7M/8F)
T3	81	66 (38M/28F)	53 (32M/21F)	13 (6M/7F)	15 (7M/8F)
Total	259	214 (122M/92 F)	169 (102M/67F)	45 (20M/25F)	45 (21M/24F)

bioRxiv preprint doi: https://doi.org/10.1101/628172; this version posted September 23, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Table 2. Top 10 Differentially Expressed Genes for Each Model (T1 vs. T2)

M1 Effect of Time Point in Patients		
Gene Symbol	Estimate	P-val FDR
TSPAN2	0,554523443	7,53143E-19
KLF6	0,162981029	2,03907E-16
MAK	0,476984074	1,20412E-15
ANTXR2	0,199530713	4,42924E-15
TMEM43	0,19700767	5,80393E-15
TREM1	0,300131404	1,1734E-14
LRRFIP1	0,178631499	1,1734E-14
NHSL2	0,253418357	2,50255E-14
ARHGEF40	0,32560393	3,23301E-14
SIPA1L1	0,228865158	3,2956E-14

M2 Response Status x Time Point		
Gene Symbol	Estimate	P-val FDR
DNER	0,19414884	0,000170023
LPCAT2	0,32653286	0,000170023
SLC10A5	-0,62752802	0,000170023
PCID2	-0,300504	0,000209042
TESC-AS1	-0,3069843	0,001180095
BECN1	0,21458155	0,001180095
IFT74	-0,43960958	0,001375265
ZNF790	-0,29221374	0,002668032
GSR	0,17871771	0,00297074
LINC01125	-0,16389193	0,00297074

M3 Patient-Control Status x Time Point		
Gene Symbol	Estimate	P-val FDR
ERN1	0,30163938	0,003013102
EZH2	0,38906547	0,003013102
SLC44A1	0,27499289	0,003013102
MEMO1	0,43613957	0,003013102
UTP11L	0,4553397	0,003013102
NSFL1C	0,2298895	0,003013102
PCTP	0,30731503	0,003013102
ZBTB16	0,53874494	0,003013102
CISH	-0,31446529	0,003456021
TRAV4	-0,4598706	0,003456021

FDR = false discovery rate

bioRxiv preprint doi: https://doi.org/10.1101/628172; this version posted September 23, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Table 3. Differential Expression in Circadian Genes after SD

		Patients [M1]	Responders vs. Non-responders [M2]	Patients vs. Controls [M3]
	Gene	T1 vs. T2	T1 vs. T2	T1 vs. T2
		P-val(β)	P-val(β)	P-val(β)
Traditional Clock Genes	PER1*	NS	0.083 FDR (0.27)	**0.045 FDR (0.32)**
	NR1D2*	NS	NS	NS
	PER3	**0.0063 FDR (-0.11)**	NS	0.065 FDR (-0.22)
	NR1D1	**0.0014 FDR (-0.12)**	0.035 unc (-0.14)	**0.036 FDR (-0.27)**
	PER2	0.038 unc (-0.059)	NS	NS
	ARNTL	**0.017 FDR (0.059)**	0.033 unc (0.096)	NS
	NPAS2	NS	NS	0.0054 unc (-0.18)
	CRY2	NS	NS	NS
	CRY1	NS	NS	NS
	CLOCK	NS	0.029 unc (-0.15)	NS
Circadian Genes	DDIT4	**1.24e-04 FDR (-0.32)**	NS	NS
	CLEC4E	**0.0023 FDR (0.20)**	**0.0483 FDR (0.42)**	**0.018 FDR (0.48)**
	FKBP5	0.037 unc (0.088)	NS	**0.028 FDR (0.38)**
	DAAM2	**0.037 FDR (0.069)**	0.020 unc (0.14)	NS
	TPST1	**1.85e-04 FDR (0.23)**	NS	**0.025 FDR (0.44)**
	IL13RA1	NS	0.0047 unc (0.19)	NS
	SMAP2	**1.16e-5 FDR (0.12)**	**0.0390 FDR (0.18)**	**0.030 FDR (0.18)**
	HNRNPDL	**1.32e-04 FDR (-0.10)**	0.0173 unc (-0.13)	**0.035 FDR (-0.18)**
	FOSL2	**2.23e-06 FDR (0.19)**	0.0046 unc (0.22)	0.0046 unc (0.22)
	FLT3	NS	NS	NS
	CDC42EP2	**4.19e-04 FDR (0.18)**	0.0071 unc (0.26)	NS
	TMEM88	**9.50e-05 FDR (0.21)**	NS	0.023 unc (0.23)
	RBM3	**7.12e-11 FDR (-0.21)**	NS	0.024 unc (-0.14)

*PER1 and NR1D2 are also on the list of circadian genes identified in Hughey et al. 2017.

FDR = false discovery rate, NS = not significant; unc = uncorrected

Blue = downregulation, Red = upregulation

Bold text indicates FDR q < 0.05

bioRxiv preprint doi: https://doi.org/10.1101/628172; this version posted September 23, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.