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Supplementary Information for

Separation of circadian- and behavior-driven metabolite rhythms in humans provides a window on peripheral oscillators and metabolism

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Materials and Methods

Clinical study. *N*=14 volunteers (10 men, 4 women) aged between 22 y and 34 y (mean ± SD: 25.8 ± 3.2 y) with a body mass index (BMI) of 25.7 ± 3.2 kg/m^2 completed the study. The participants were randomized to either the simulated day shift condition (7 participants) or the simulated night shift condition (7 participants); see Fig. S1. There was a small age difference between the two groups (day shift condition: 24.0 ± 2.2 y; night shift condition: 27.6 ± 3.2 y; t_{12}=2.4, p=0.031). The groups were not significantly different with regard to BMI (day shift condition: 25.9 ± 3.4 kg/m^2; night shift condition: 25.6 ± 3.3 kg/m^2; t_{12}=0.14, p=0.89) or sex distribution (day shift condition: 4 men; night shift condition: 6 men; χ_1^2=1.4, p=0.24).

Participants were screened to be physically and psychologically healthy with no medical or drug treatment, as verified by physical examination, blood chemistry, urinalysis, and questionnaires. They reported good habitual sleep, between 6 and 10 hours in duration and habitually getting up between 06:00 and 09:00, and were no extreme morning- or evening-types as assessed by questionnaire (Composite Scale of Morningness). They showed no evidence of any sleep or circadian disorders as assessed by nocturnal polysomnography (1) and validated questionnaires (Pittsburgh Sleep Quality Index, Sleep Disorders Questionnaire, Epworth Sleepiness Scale). They had no history of drug or alcohol abuse, were free of traces of alcohol and drugs as assessed by drug screen and breathalyzer, and did not smoke. They were not involved in shift work within three months of entering the study, and did not travel across time zones within one month of entering the study. Women were not pregnant as assessed by blood-based assay.

Participants maintained a regular sleep/wake schedule in the week prior to the in-laboratory experiment, with bedtimes and wake times within 30 minutes from their self-reported habitual schedule. This was confirmed by means of wrist actigraphy, sleep diary, and participants calling a time-stamped voice mail recorder upon waking and before going to bed. In the week prior to the study, participants were requested to abstain from alcohol and caffeine.

Targeted metabolomics analysis. Although targeted metabolomics does not provide a complete assessment of the entire metabolome, it covers important metabolite classes, and the results obtained are quantifiable and highly reproducible across laboratories (2). Targeted metabolomics is also well suited for high throughput of large number of samples as generated by time-series circadian studies.

Metabolites were quantified in the plasma samples collected at 3-h intervals during the 24-h constant routine using the Absolute***IDQ*** p180 targeted metabolomics kit (Biocrates Life Sciences AG, Innsbruck, Austria) and a Waters Xevo TQ-S mass spectrometer coupled to an Acquity UPLC system (Waters Corporation, Milford, MA, USA). Plasma samples (10 µl) were prepared according to the manufacturer's instructions adding several stable isotope–labeled standards to the samples prior to the derivatization and extraction steps. Using LC/MS (liquid chromatography/mass spectrometry), 184 metabolites from 5 different compound classes (acylcarnitines, amino acids, biogenic amines, glycerophospholipids, and sphingolipids) were quantified.

The samples were run on 2×96-well plates. Sample order was randomized and three levels of quality control (QC) were run on each plate. Data were normalized between the plates using the results of quality control level 2 (QC2) repeats across the plate (*n*=4) and between plates (*n*=2) using Biocrates METIDQ software (QC2 correction). Metabolites where >25% samples were below the limit of detection, below the lower limit of quantification, above the upper limit of quantification, or blank out of range, or where the QC2 coefficient of variance was >30%, were excluded. This was the case for 52 metabolites. The remaining 132 quantified metabolites comprised 10 acylcarnitines, 20 amino acids, 7 biogenic amines, 81 glycerophospholipids, and 14 sphingolipids.

Plasma melatonin. Melatonin levels were measured in the plasma samples collected hourly at baseline (18:30–21:30) and during the constant routine (18:30–01:30) using RIA (IB88111, IBL, Minneapolis, MN, USA) performed in duplicate by KMI Diagnostics, Inc. (Minneapolis, MN, USA).

Plasma cortisol. Cortisol levels were measured in the plasma samples collected at 3-h intervals during the 24-h constant routine using ELISA (ADI-900-097, Enzo Life Sciences, Farmingdale, NY, USA) following the manufacturer's instructions. Assays were performed in triplicate. Standards were used as per the kit manual. Analyses were performed using MyAssays software as recommended (https://www.myassays.com/corticosterone-(enzo).assay).

PER3 expression. *PER3* transcript levels were measured in the plasma samples collected at 3-h intervals during the 24-h constant routine using nCounter multiplexed assay (NanoString Technologies, Seattle, WA, USA). RNA was measured directly with no amplification or other enzymatic processing. 100 ng of total RNA was used for the assay. Analysis was performed using nSolver 3.0 software (NanoString Technologies, Seattle, WA, USA). Genes with an expectancy of less than 10 counts with 90% occurrence or more were removed. Background values were subtracted and data were normalized with overall geometric mean of internal control genes.

Statistical analysis. Cosinor analysis to investigate 24-h rhythmicity in all of the metabolites, cortisol, and *PER3* expression, was performed across all participants in both conditions using linear mixed-effects regression (3). Regression coefficients for mesor (center level), acrophase (timing of peak), and amplitude (difference between mesor and level at acrophase) were assessed (Table S1) and used for statistical testing. Rhythm significance in each of the two conditions was determined by testing amplitude against zero using t test, with a one-sided type I error threshold of 0.05. 95% confidence intervals for acrophase were computed using the delta method. Differences between conditions in acrophase, amplitude and mesor were evaluated using t test, with a two-sided type I error threshold of 0.05.

The cosinor analyses for metabolites were repeated after removal of data from the first 3 h of the 24-h constant routine period, in order to verify robustness to any transient effects from physiological adjustment to the constant routine procedures. This resulted in minor differences in acrophase estimates (mean ± SD: 38 ± 34 min) and did not substantively affect any of the findings. Separately, the cosinor analyses for metabolites were repeated with time awake added as a regression term. Out of the 132 metabolites analyzed, 115 did not show a significant effect of time awake. For the 17 metabolites that did show a significant effect of time awake, the levels of 7 metabolites decreased and the levels of 10 metabolites increased across the 24-h constant routine. In all cases, the total change over time was small relative to the 24-h overall average. The constant routine procedure and the increasing sleep pressure associated with 24-h sustained wakefulness did not produce any substantive confounds in the metabolites' temporal profiles.

The timing of dim light melatonin onset (DLMO) was analyzed with non-linear mixed-effects regression of a Taylor series approximation of the melatonin curve (Fig. S2). Differences between conditions were evaluated using t test. Differences between baseline and constant routine were evaluated using paired-sample t test. Although this methodology does not require the definition of a threshold to estimate timing differences, we used a conventional 10 pg/ml threshold (4) to define the DLMO.

Principal component analysis (PCA) was based on scree plot to determine how many factors to retain, and used varimax rotation to assess factor loadings. All statistical analyses were implemented in SAS version 9.4.

Pathway analyses were performed for the day shift and night shift conditions separately (Tables S3, S4) using the MetaboAnalyst 3.0 database (5). Enriched pathways were identified using a false discovery rate (FDR) threshold of 0.05.

Table S1. Statistical results of cosinor analyses. Statistically significant differences from zero (p<0.05) are indicated by bold font for amplitude (orange, purple) and for difference between conditions in amplitude (black), mesor (gray, italicized), and phase (red).

	Day Shift Condition			*Night Shift Condition*			*Difference between Conditions*		
Metabolite	*Amplitude ± SE*	*Mesor ± SE*	*Acrophase ± SE*	*Amplitude ± SE*	*Mesor ± SE*	*Acrophase ± SE*	*Amplitude Δ ± SE*	*Mesor Δ ± SE*	*Acrophase Δ ± SE*
PC aa C30:0	**0.283 ± 0.054**	1.446 ± 0.126	11:20 ± 00:42	**0.222 ± 0.054**	1.397 ± 0.126	23:44 ± 00:53	-0.062 ± 0.076	-0.049 ± 0.178	**-11:36 ± 01:08**
PC ae C34:3	**0.387 ± 0.143**	4.012 ± 0.390	13:02 ± 01:22	**0.331 ± 0.143**	3.904 ± 0.389	23:29 ± 01:35	-0.056 ± 0.203	-0.108 ± 0.551	**10:26 ± 02:06**
PC aa C36:6	**0.035 ± 0.014**	0.357 ± 0.041	13:44 ± 01:29	**0.049 ± 0.014**	0.374 ± 0.041	23:43 ± 01:01	0.015 ± 0.020	0.017 ± 0.058	**09:59 ± 01:48**
PC aa C32:1	**0.507 ± 0.161**	3.910 ± 0.506	12:51 ± 01:11	**1.054 ± 0.161**	4.373 ± 0.506	00:44 ± 00:33	**0.547 ± 0.228**	0.463 ± 0.715	**11:52 ± 01:18**
PC ae C34:0	**0.146 ± 0.039**	0.918 ± 0.066	11:53 ± 00:59	**0.091 ± 0.039**	0.985 ± 0.066	01:35 ± 01:35	-0.055 ± 0.055	0.068 ± 0.093	**-10:17 ± 01:52**
PC ae C32:1	0.068 ± 0.040	1.169 ± 0.085	13:48 ± 02:13	0.056 ± 0.040	1.195 ± 0.085	23:27 ± 02:36	-0.011 ± 0.057	0.026 ± 0.120	**09:39 ± 03:25**
PC ae C34:2	**0.572 ± 0.229**	6.642 ± 0.422	13:03 ± 01:29	0.410 ± 0.228	6.341 ± 0.422	01:22 ± 02:03	-0.162 ± 0.323	-0.301 ± 0.597	**-11:41 ± 02:32**
PC ae C34:1	0.244 ± 0.160	4.708 ± 0.261	13:08 ± 02:27	**0.406 ± 0.161**	4.766 ± 0.260	00:36 ± 01:27	0.162 ± 0.227	0.058 ± 0.368	**11:28 ± 02:51**
PC aa C34:3	0.614 ± 0.318	9.415 ± 0.675	12:28 ± 01:55	**1.183 ± 0.317**	8.647 ± 0.675	01:24 ± 00:59	0.569 ± 0.449	-0.768 ± 0.954	**-11:03 ± 02:10**
PC aa C34:4	**0.166 ± 0.036**	1.043 ± 0.085	12:52 ± 00:48	**0.171 ± 0.037**	0.955 ± 0.085	00:26 ± 00:47	0.005 ± 0.052	-0.088 ± 0.120	**11:33 ± 01:07**
PC aa C32:0	**0.930 ± 0.291**	7.324 ± 0.463	10:56 ± 01:10	**0.748 ± 0.290**	7.614 ± 0.463	01:38 ± 01:26	-0.182 ± 0.410	0.290 ± 0.655	**-09:17 ± 01:51**
PC ae C36:2	0.490 ± 0.337	10.310 ± 0.602	16:13 ± 02:40	0.511 ± 0.333	9.905 ± 0.602	04:40 ± 02:33	0.021 ± 0.473	-0.405 ± 0.852	**-11:32 ± 03:41**
PC ae C38:6	0.174 ± 0.122	3.185 ± 0.273	14:57 ± 02:40	0.194 ± 0.122	3.709 ± 0.273	22:04 ± 02:21	0.020 ± 0.172	0.524 ± 0.387	07:07 ± 03:33
PC ae C36:5	0.455 ± 0.239	6.485 ± 0.634	13:21 ± 01:57	**0.547 ± 0.239**	7.193 ± 0.634	23:30 ± 01:36	0.092 ± 0.338	0.707 ± 0.897	**10:08 ± 02:32**
PC ae C36:1	0.242 ± 0.235	6.938 ± 0.410	15:56 ± 03:45	0.396 ± 0.239	7.426 ± 0.410	01:34 ± 02:14	0.154 ± 0.335	0.489 ± 0.580	**09:37 ± 04:22**
PC ae C36:0	**0.038 ± 0.018**	0.444 ± 0.031	09:45 ± 01:47	0.011 ± 0.017	0.460 ± 0.031	06:12 ± 06:16	-0.027 ± 0.025	0.016 ± 0.044	-03:32 ± 06:32
PC ae C32:2	0.019 ± 0.011	0.330 ± 0.022	15:23 ± 02:11	0.021 ± 0.011	0.353 ± 0.022	22:56 ± 01:54	0.002 ± 0.015	0.024 ± 0.031	**07:32 ± 02:54**
PC aa C32:3	**0.035 ± 0.008**	0.250 ± 0.013	11:49 ± 00:53	**0.035 ± 0.008**	0.244 ± 0.013	23:32 ± 00:53	0.000 ± 0.012	-0.006 ± 0.018	**11:42 ± 01:15**
SM C18:0	**1.869 ± 0.939**	23.392 ± 1.664	12:52 ± 01:52	**2.286 ± 0.939**	26.502 ± 1.663	00:38 ± 01:30	0.417 ± 1.328	3.111 ± 2.352	**11:46 ± 02:24**
PC ae C36:3	0.237 ± 0.141	4.173 ± 0.255	14:27 ± 02:15	0.237 ± 0.143	4.057 ± 0.254	00:04 ± 02:12	0.000 ± 0.201	-0.116 ± 0.360	**09:36 ± 03:09**
PC aa C34:1	3.983 ± 3.303	103.063 ± 5.529	16:54 ± 03:14	**8.304 ± 3.321**	107.107 ± 5.525	03:08 ± 01:31	4.321 ± 4.683	4.044 ± 7.816	**10:13 ± 03:35**
SM C18:1	**0.895 ± 0.421**	10.741 ± 0.782	13:06 ± 01:45	**0.975 ± 0.421**	11.770 ± 0.782	00:44 ± 01:35	0.081 ± 0.595	1.030 ± 1.106	**11:38 ± 02:22**
PC aa C40:6	0.774 ± 0.551	14.210 ± 1.873	17:52 ± 02:47	0.920 ± 0.566	18.167 ± 1.873	23:49 ± 02:15	0.146 ± 0.790	3.958 ± 2.649	05:57 ± 03:35
PC aa C38:6	1.961 ± 1.526	38.695 ± 4.470	20:02 ± 03:00	1.624 ± 1.557	48.038 ± 4.469	00:25 ± 03:31	-0.337 ± 2.180	9.343 ± 6.321	04:22 ± 04:38
PC ae C30:0	**0.019 ± 0.007**	0.204 ± 0.014	14:03 ± 01:22	**0.021 ± 0.007**	0.201 ± 0.014	22:54 ± 01:14	0.002 ± 0.010	-0.003 ± 0.019	**08:50 ± 01:50**
SM C16:0	5.082 ± 4.232	110.256 ± 7.735	13:30 ± 03:07	4.851 ± 4.244	116.946 ± 7.730	00:38 ± 03:13	-0.231 ± 5.994	6.689 ± 10.935	**11:07 ± 04:29**
PC ae C38:0	0.056 ± 0.037	1.021 ± 0.112	22:11 ± 02:31	0.035 ± 0.037	1.107 ± 0.112	01:52 ± 04:00	-0.021 ± 0.053	0.087 ± 0.158	03:41 ± 04:44
PC aa C38:0	0.065 ± 0.066	1.642 ± 0.199	14:43 ± 03:54	0.089 ± 0.065	1.964 ± 0.199	20:23 ± 02:50	0.025 ± 0.093	0.321 ± 0.281	05:40 ± 04:49
PC ae C42:2	0.004 ± 0.011	0.312 ± 0.025	17:29 ± 10:28	0.009 ± 0.011	0.338 ± 0.025	02:42 ± 04:37	0.005 ± 0.016	0.026 ± 0.036	09:13 ± 11:27
SM (OH) C22:1	1.127 ± 1.087	26.956 ± 1.742	13:45 ± 03:37	1.572 ± 1.091	30.504 ± 1.741	00:43 ± 02:33	0.445 ± 1.540	3.548 ± 2.463	**10:58 ± 04:25**
SM (OH) C16:1	0.203 ± 0.137	3.415 ± 0.228	14:06 ± 02:32	0.267 ± 0.138	3.859 ± 0.228	00:25 ± 01:54	0.064 ± 0.194	0.444 ± 0.322	**10:19 ± 03:10**
SM (OH) C22:2	1.406 ± 1.066	28.123 ± 1.764	15:12 ± 02:54	1.817 ± 1.082	30.791 ± 1.762	00:25 ± 02:11	0.411 ± 1.518	2.668 ± 2.493	**09:12 ± 03:38**
PC aa C36:0	**0.323 ± 0.108**	0.945 ± 0.190	09:25 ± 01:15	**0.389 ± 0.109**	1.648 ± 0.190	00:48 ± 01:01	0.065 ± 0.153	***0.704 ± 0.269***	**-08:37 ± 01:37**
SM C16:1	0.688 ± 0.603	16.426 ± 0.970	13:55 ± 03:17	0.594 ± 0.606	16.004 ± 0.969	00:30 ± 03:45	-0.094 ± 0.855	-0.422 ± 1.371	10:35 ± 04:59
PC ae C40:1	0.012 ± 0.033	0.851 ± 0.091	08:55 ± 10:08	0.035 ± 0.033	0.838 ± 0.091	09:37 ± 03:35	0.022 ± 0.047	-0.012 ± 0.129	00:41 ± 10:45
SM (OH) C14:1	0.275 ± 0.216	5.576 ± 0.357	14:03 ± 02:57	0.254 ± 0.218	5.794 ± 0.357	23:43 ± 03:08	-0.020 ± 0.307	0.218 ± 0.505	**09:39 ± 04:19**
PC aa C36:1	1.447 ± 1.323	32.326 ± 2.741	19:18 ± 03:34	1.589 ± 1.355	31.253 ± 2.739	11:34 ± 03:08	0.142 ± 1.894	-1.074 ± 3.875	-07:44 ± 04:45
PC aa C36:5	**0.495 ± 0.243**	6.735 ± 0.627	15:53 ± 01:54	**0.895 ± 0.246**	7.804 ± 0.627	01:53 ± 01:01	0.400 ± 0.346	1.069 ± 0.886	**10:00 ± 02:09**
Tyr	1.358 ± 1.956	58.837 ± 2.659	10:59 ± 05:19	2.573 ± 1.887	59.846 ± 2.659	06:03 ± 02:54	1.214 ± 2.718	1.009 ± 3.760	-04:55 ± 06:03
PC aa C36:3	2.551 ± 2.542	81.641 ± 4.062	20:02 ± 03:51	3.936 ± 2.497	78.492 ± 4.059	05:41 ± 02:31	1.384 ± 3.563	-3.149 ± 5.742	09:38 ± 04:36
PC aa C36:4	3.047 ± 3.576	112.319 ± 4.458	15:39 ± 04:31	**8.412 ± 3.581**	118.737 ± 4.452	02:49 ± 01:37	5.365 ± 5.061	6.419 ± 6.301	**11:09 ± 04:48**
PC ae C40:6	0.106 ± 0.094	2.414 ± 0.190	16:06 ± 03:25	0.138 ± 0.096	2.995 ± 0.190	22:51 ± 02:33	0.032 ± 0.134	0.581 ± 0.269	06:44 ± 04:16
PC aa C36:2	4.188 ± 5.069	114.921 ± 5.231	09:10 ± 04:36	**15.580 ± 5.040**	116.477 ± 5.221	02:56 ± 01:14	11.393 ± 7.148	1.555 ± 7.390	-06:14 ± 04:46
PC aa C42:6	0.004 ± 0.008	0.184 ± 0.015	17:52 ± 08:06	0.006 ± 0.008	0.214 ± 0.015	01:43 ± 04:46	0.002 ± 0.011	0.030 ± 0.021	07:50 ± 09:24

Metabolite	*Day Shift Condition*			*Night Shift Condition*			*Difference between Conditions*		
	Amplitude ± SE	*Mesor ± SE*	*Acrophase ± SE*	*Amplitude ± SE*	*Mesor ± SE*	*Acrophase ± SE*	*Amplitude Δ ± SE*	*Mesor Δ ± SE*	*Acrophase Δ ± SE*
PC aa C28:1	0.064 ± 0.046	1.534 ± 0.088	14:46 ± 02:45	0.052 ± 0.047	1.495 ± 0.088	00:04 ± 03:19	-0.012 ± 0.066	-0.040 ± 0.125	09:18 ± 04:19
SM C24:0	0.714 ± 1.024	26.268 ± 2.074	12:05 ± 05:19	0.743 ± 1.008	26.950 ± 2.073	02:43 ± 05:09	0.029 ± 1.437	0.682 ± 2.932	-09:22 ± 07:24
PC aa C38:3	1.903 ± 0.984	29.542 ± 3.120	18:42 ± 02:01	1.608 ± 1.003	32.467 ± 3.120	01:48 ± 02:19	-0.295 ± 1.406	2.925 ± 4.412	**07:06 ± 03:05**
LysoPC a C28:1	0.018 ± 0.010	0.305 ± 0.023	16:45 ± 02:01	0.019 ± 0.010	0.308 ± 0.023	22:30 ± 01:55	0.000 ± 0.014	0.002 ± 0.033	05:45 ± 02:47
PC aa C34:2	8.425 ± 7.822	134.105 ± 6.959	07:55 ± 03:36	**23.568 ± 7.873**	140.496 ± 6.942	02:36 ± 01:15	15.143 ± 11.098	6.391 ± 9.830	-05:18 ± 03:49
SM C20:2	**0.064 ± 0.023**	0.550 ± 0.033	12:21 ± 01:19	**0.057 ± 0.023**	0.551 ± 0.032	00:36 ± 01:27	-0.006 ± 0.032	0.001 ± 0.046	**-11:44 ± 01:57**
PC ae C42:1	0.015 ± 0.010	0.262 ± 0.023	11:22 ± 02:36	0.015 ± 0.010	0.259 ± 0.023	02:46 ± 02:33	0.000 ± 0.014	-0.003 ± 0.032	**-08:35 ± 03:38**
Lys	**15.622 ± 4.876**	153.876 ± 8.735	12:25 ± 01:08	4.336 ± 4.838	145.122 ± 8.735	22:09 ± 04:10	-11.286 ± 6.869	-8.754 ± 12.353	**09:43 ± 04:19**
PC ae C38:2	**0.064 ± 0.027**	0.646 ± 0.080	15:32 ± 01:39	0.032 ± 0.027	0.464 ± 0.080	05:41 ± 03:24	-0.032 ± 0.038	-0.182 ± 0.113	**-09:51 ± 03:47**
PC ae C30:2	0.003 ± 0.002	0.062 ± 0.002	15:41 ± 03:14	0.002 ± 0.002	0.064 ± 0.002	21:45 ± 03:19	0.000 ± 0.003	0.002 ± 0.003	06:04 ± 04:39
C9	0.001 ± 0.002	0.024 ± 0.002	05:56 ± 12:06	**0.007 ± 0.002**	0.028 ± 0.002	02:10 ± 01:18	0.006 ± 0.003	0.003 ± 0.002	-03:45 ± 12:11
C3-DC (C4-OH)	0.003 ± 0.005	0.027 ± 0.005	08:55 ± 05:16	**0.013 ± 0.005**	0.033 ± 0.005	04:41 ± 01:28	0.009 ± 0.007	0.006 ± 0.007	-04:13 ± 05:28
C14:2	0.001 ± 0.002	0.017 ± 0.001	06:20 ± 06:17	**0.003 ± 0.002**	0.020 ± 0.001	04:13 ± 01:59	0.002 ± 0.002	0.003 ± 0.002	-02:06 ± 06:35
LysoPC a C20:4	**0.974 ± 0.228**	5.755 ± 0.489	20:24 ± 00:54	0.073 ± 0.224	6.080 ± 0.488	16:51 ± 12:10	**-0.902 ± 0.320**	0.325 ± 0.691	-03:33 ± 12:12
LysoPC a C17:0	**0.123 ± 0.057**	1.373 ± 0.078	19:16 ± 01:48	0.079 ± 0.059	1.511 ± 0.077	00:13 ± 02:42	-0.044 ± 0.082	0.138 ± 0.110	04:57 ± 03:15
PC ae C38:4	0.396 ± 0.288	7.835 ± 0.507	15:36 ± 02:47	0.398 ± 0.291	9.105 ± 0.507	01:33 ± 02:43	0.001 ± 0.409	1.270 ± 0.717	**09:56 ± 03:54**
C0	0.842 ± 1.045	30.552 ± 1.708	11:30 ± 04:34	0.705 ± 1.008	32.497 ± 1.708	05:06 ± 05:39	-0.137 ± 1.452	1.945 ± 2.416	-06:23 ± 07:16
Leu	**7.872 ± 3.622**	110.720 ± 4.755	19:48 ± 01:47	4.403 ± 3.594	111.318 ± 4.755	05:32 ± 03:14	-3.470 ± 5.102	0.597 ± 6.725	**09:43 ± 03:42**
Ile	**6.308 ± 2.210**	70.260 ± 2.481	20:16 ± 01:21	**5.073 ± 2.207**	68.619 ± 2.481	08:06 ± 01:41	-1.235 ± 3.123	-1.640 ± 3.508	**11:49 ± 02:10**
LysoPC a C20:3	**0.464 ± 0.083**	2.013 ± 0.144	20:24 ± 00:41	0.042 ± 0.082	1.976 ± 0.143	19:18 ± 07:40	**-0.422 ± 0.117**	-0.037 ± 0.203	-01:05 ± 07:41
PC ae C40:4	0.065 ± 0.054	1.480 ± 0.078	14:46 ± 03:07	0.081 ± 0.054	1.710 ± 0.078	00:42 ± 02:27	0.016 ± 0.076	0.230 ± 0.110	**09:56 ± 03:58**
PC aa C38:4	2.191 ± 2.239	68.228 ± 4.628	17:52 ± 04:00	**4.498 ± 2.251**	77.889 ± 4.625	03:22 ± 01:55	2.307 ± 3.175	9.661 ± 6.543	09:30 ± 04:27
Val	10.897 ± 6.738	227.412 ± 8.512	13:51 ± 02:18	6.083 ± 6.620	209.350 ± 8.512	03:41 ± 04:12	-4.814 ± 9.446	-18.063 ± 12.037	-10:09 ± 04:48
LysoPC a C16:0	4.658 ± 2.638	66.578 ± 3.512	18:36 ± 02:13	3.444 ± 2.707	69.452 ± 3.508	00:44 ± 02:53	-1.214 ± 3.780	2.875 ± 4.963	06:08 ± 03:39
PC ae C38:5	0.524 ± 0.335	9.572 ± 0.485	14:10 ± 02:24	0.531 ± 0.338	10.038 ± 0.484	23:47 ± 02:20	0.007 ± 0.476	0.466 ± 0.685	**09:36 ± 03:21**
LysoPC a C18:0	**1.571 ± 0.780**	19.623 ± 1.346	19:24 ± 01:56	0.686 ± 0.798	20.393 ± 1.345	00:45 ± 04:17	-0.885 ± 1.116	0.770 ± 1.903	05:21 ± 04:42
PC aa C38:5	1.304 ± 0.998	27.671 ± 1.821	18:49 ± 02:59	1.797 ± 1.005	31.887 ± 1.820	03:00 ± 02:08	0.493 ± 1.417	4.216 ± 2.574	**08:11 ± 03:40**
Phe	**3.636 ± 1.834**	59.819 ± 1.771	17:58 ± 02:00	1.469 ± 1.872	57.943 ± 1.771	02:56 ± 04:51	-2.167 ± 2.620	-1.875 ± 2.505	08:58 ± 05:15
PC ae C36:4	0.613 ± 0.319	8.794 ± 0.527	13:29 ± 01:56	**0.631 ± 0.320**	9.430 ± 0.526	23:57 ± 01:51	0.018 ± 0.452	0.637 ± 0.745	**10:28 ± 02:41**
PC ae C40:5	0.099 ± 0.087	2.372 ± 0.120	15:30 ± 03:22	0.130 ± 0.089	2.798 ± 0.120	00:21 ± 02:30	0.031 ± 0.125	*0.426 ± 0.170*	08:50 ± 04:12
Trp	2.420 ± 1.859	56.493 ± 2.664	22:01 ± 02:52	**4.208 ± 1.871**	56.037 ± 2.664	01:02 ± 01:38	1.788 ± 2.638	-0.455 ± 3.768	03:00 ± 03:18
Kynurenine	**0.118 ± 0.055**	1.646 ± 0.087	10:03 ± 01:43	0.099 ± 0.053	1.686 ± 0.087	05:09 ± 02:07	-0.019 ± 0.076	0.040 ± 0.123	-04:54 ± 02:44
His	**4.665 ± 2.318**	78.016 ± 2.460	15:08 ± 01:54	0.881 ± 2.334	77.704 ± 2.460	02:26 ± 10:00	-3.784 ± 3.289	-0.312 ± 3.478	11:17 ± 10:11
LysoPC a C18:1	**2.545 ± 0.558**	14.465 ± 0.648	21:27 ± 00:49	**1.158 ± 0.561**	13.529 ± 0.647	13:25 ± 01:47	-1.387 ± 0.791	-0.936 ± 0.916	**-08:01 ± 01:58**
PC aa C40:4	0.087 ± 0.090	2.603 ± 0.282	16:34 ± 04:03	0.148 ± 0.091	2.844 ± 0.282	02:44 ± 02:20	0.061 ± 0.128	0.240 ± 0.399	10:09 ± 04:41
C18:2	0.002 ± 0.002	0.030 ± 0.002	18:09 ± 02:55	0.002 ± 0.002	0.032 ± 0.002	00:31 ± 02:32	0.000 ± 0.002	0.002 ± 0.002	06:21 ± 03:52
PC ae C44:6	0.038 ± 0.026	0.734 ± 0.046	16:20 ± 02:38	0.032 ± 0.027	0.841 ± 0.046	23:39 ± 03:01	-0.006 ± 0.037	0.108 ± 0.066	07:18 ± 04:00
PC aa C40:5	0.262 ± 0.211	5.692 ± 0.512	17:46 ± 03:09	**0.461 ± 0.215**	6.984 ± 0.512	01:59 ± 01:44	0.199 ± 0.301	1.292 ± 0.724	**08:13 ± 03:36**
PC ae C42:5	0.052 ± 0.040	1.007 ± 0.046	16:47 ± 02:58	0.052 ± 0.040	1.195 ± 0.045	22:26 ± 02:52	0.000 ± 0.057	*0.187 ± 0.064*	05:39 ± 04:07
PC ae C38:3	0.173 ± 0.121	3.599 ± 0.232	17:27 ± 02:44	0.210 ± 0.123	3.967 ± 0.232	01:26 ± 02:11	0.037 ± 0.173	0.368 ± 0.328	**07:59 ± 03:29**
C2	0.216 ± 0.129	3.388 ± 0.159	22:11 ± 02:14	**0.478 ± 0.129**	3.970 ± 0.158	10:43 ± 01:00	0.262 ± 0.182	*0.582 ± 0.224*	**-11:27 ± 02:27**
Met	0.872 ± 0.837	22.893 ± 0.844	13:30 ± 03:34	1.029 ± 0.821	24.527 ± 0.844	20:17 ± 03:05	0.157 ± 1.172	1.635 ± 1.194	06:47 ± 04:43
LysoPC a C16:1	**0.150 ± 0.070**	1.792 ± 0.096	18:57 ± 01:49	0.131 ± 0.072	1.930 ± 0.096	00:39 ± 02:01	-0.019 ± 0.100	0.139 ± 0.135	05:41 ± 02:43
PC ae C44:5	0.038 ± 0.032	0.880 ± 0.057	16:36 ± 03:16	0.040 ± 0.032	1.018 ± 0.057	23:02 ± 03:01	0.002 ± 0.045	0.138 ± 0.080	06:26 ± 04:27
SDMA	0.005 ± 0.015	0.460 ± 0.020	22:58 ± 10:36	0.021 ± 0.015	0.454 ± 0.020	22:20 ± 02:39	0.016 ± 0.022	-0.006 ± 0.028	-00:38 ± 10:55
LysoPC a C18:2	**6.531 ± 1.579**	40.562 ± 1.745	19:44 ± 00:56	**4.109 ± 1.615**	33.358 ± 1.742	11:56 ± 01:26	-2.422 ± 2.258	*-7.204 ± 2.466*	**-07:47 ± 01:43**
PC aa C42:4	0.005 ± 0.006	0.161 ± 0.011	01:03 ± 04:35	0.007 ± 0.006	0.160 ± 0.011	11:31 ± 03:10	0.002 ± 0.009	-0.001 ± 0.016	10:28 ± 05:34
Sarcosine	**1.103 ± 0.175**	4.727 ± 0.288	20:50 ± 00:36	**1.381 ± 0.176**	4.913 ± 0.288	21:47 ± 00:28	0.279 ± 0.248	0.186 ± 0.407	00:57 ± 00:46

Metabolite	*Day Shift Condition*			*Night Shift Condition*			*Difference between Conditions*		
	Amplitude ± SE	*Mesor ± SE*	*Acrophase ± SE*	*Amplitude ± SE*	*Mesor ± SE*	*Acrophase ± SE*	*Amplitude Δ ± SE*	*Mesor Δ ± SE*	*Acrophase Δ ± SE*
Pro	**35.570 ± 7.014**	203.241 ± 13.280	11:51 ± 00:43	**16.491 ± 6.994**	174.900 ± 13.280	22:54 ± 01:34	-19.079 ± 9.905	-28.341 ± 18.781	**11:02 ± 01:43**
Creatinine	3.609 ± 8.006	126.698 ± 9.725	02:28 ± 08:23	6.581 ± 7.809	134.485 ± 9.725	06:02 ± 04:42	2.972 ± 11.184	7.786 ± 13.754	03:33 ± 09:37
LysoPC a C24:0	0.005 ± 0.005	0.122 ± 0.006	19:05 ± 03:27	0.003 ± 0.005	0.130 ± 0.006	21:09 ± 05:18	-0.002 ± 0.006	0.008 ± 0.009	02:03 ± 06:19
LysoPC a C28:0	**0.015 ± 0.006**	0.150 ± 0.010	16:25 ± 01:35	0.011 ± 0.006	0.161 ± 0.010	22:51 ± 01:59	-0.003 ± 0.009	0.011 ± 0.014	**06:25 ± 02:32**
C5-DC (C6-OH)	0.000 ± 0.001	0.010 ± 0.001	15:28 ± 05:48	**0.002 ± 0.001**	0.011 ± 0.001	05:13 ± 01:37	0.001 ± 0.001	0.001 ± 0.001	-10:15 ± 06:01
C14:1	0.002 ± 0.002	0.031 ± 0.002	19:23 ± 03:43	0.001 ± 0.002	0.034 ± 0.002	06:02 ± 04:51	0.000 ± 0.002	0.003 ± 0.002	10:38 ± 06:07
LysoPC a C26:1	**0.011 ± 0.004**	0.102 ± 0.004	18:27 ± 01:23	0.008 ± 0.004	0.116 ± 0.004	00:20 ± 01:56	-0.003 ± 0.006	*0.013 ± 0.006*	**05:52 ± 02:23**
C7-DC	0.001 ± 0.002	0.016 ± 0.002	14:21 ± 07:48	0.003 ± 0.002	0.016 ± 0.002	08:15 ± 02:19	0.002 ± 0.002	0.001 ± 0.002	-06:05 ± 08:08
LysoPC a C26:0	**0.023 ± 0.008**	0.176 ± 0.011	18:05 ± 01:20	**0.027 ± 0.008**	0.194 ± 0.011	23:10 ± 01:05	0.004 ± 0.011	0.018 ± 0.015	**05:05 ± 01:43**
Gly	15.780 ± 8.286	259.371 ± 17.728	13:50 ± 01:57	4.999 ± 8.306	271.632 ± 17.728	13:31 ± 06:10	-10.781 ± 11.732	12.261 ± 25.071	-00:18 ± 06:28
Asn	1.589 ± 2.247	58.917 ± 3.473	22:03 ± 05:17	4.095 ± 2.252	64.240 ± 3.473	10:19 ± 02:02	2.506 ± 3.181	5.323 ± 4.911	-11:44 ± 05:40
PC aa C42:0	0.016 ± 0.014	0.334 ± 0.027	16:27 ± 03:14	0.017 ± 0.014	0.427 ± 0.027	20:54 ± 03:01	0.001 ± 0.019	*0.093 ± 0.039*	04:27 ± 04:25
Arg	**8.037 ± 3.211**	92.811 ± 7.771	18:33 ± 01:35	**6.480 ± 3.330**	98.089 ± 7.771	12:42 ± 01:53	-1.557 ± 4.626	5.278 ± 10.990	**-05:51 ± 02:28**
Thr	**8.757 ± 4.405**	122.990 ± 6.769	11:09 ± 01:51	3.478 ± 4.263	147.150 ± 6.769	19:15 ± 04:49	-5.279 ± 6.130	*24.160 ± 9.573*	08:05 ± 05:10
PC aa C42:1	0.007 ± 0.007	0.185 ± 0.017	17:52 ± 03:57	0.008 ± 0.007	0.231 ± 0.017	18:44 ± 03:48	0.000 ± 0.010	0.047 ± 0.024	00:52 ± 05:29
Ser	3.685 ± 3.420	99.101 ± 7.197	21:51 ± 03:29	5.626 ± 3.415	112.155 ± 7.197	09:39 ± 02:17	1.940 ± 4.833	13.054 ± 10.178	**11:48 ± 04:10**
PC ae C40:2	0.052 ± 0.035	0.981 ± 0.075	15:36 ± 02:37	0.061 ± 0.036	1.130 ± 0.075	22:53 ± 02:09	0.009 ± 0.050	0.149 ± 0.106	07:17 ± 03:23
Gln	**56.291 ± 20.540**	659.548 ± 36.599	15:30 ± 01:24	28.488 ± 20.662	674.291 ± 36.599	14:55 ± 02:45	-27.803 ± 29.134	14.743 ± 51.759	-00:35 ± 03:06
PC ae C42:3	0.008 ± 0.017	0.484 ± 0.036	16:40 ± 08:36	0.006 ± 0.017	0.508 ± 0.036	22:19 ± 09:51	-0.001 ± 0.024	0.024 ± 0.051	05:39 ± 13:04
SM C26:1	0.029 ± 0.018	0.400 ± 0.041	13:01 ± 02:21	0.033 ± 0.018	0.459 ± 0.041	02:41 ± 02:03	0.004 ± 0.025	0.059 ± 0.058	**-10:20 ± 03:07**
PC ae C44:3	0.000 ± 0.002	0.055 ± 0.004	13:10 ± 21:20	**0.005 ± 0.002**	0.061 ± 0.004	23:34 ± 01:50	0.004 ± 0.003	0.006 ± 0.005	10:24 ± 21:25
SM C26:0	0.003 ± 0.010	0.245 ± 0.019	12:01 ± 11:10	0.007 ± 0.010	0.261 ± 0.019	21:30 ± 05:53	0.003 ± 0.014	0.017 ± 0.026	09:29 ± 12:37
SM (OH) C24:1	0.061 ± 0.059	1.505 ± 0.099	14:31 ± 03:41	0.077 ± 0.060	1.684 ± 0.099	01:09 ± 02:52	0.016 ± 0.084	0.179 ± 0.140	**10:37 ± 04:41**
Orn	**3.384 ± 1.525**	47.645 ± 3.239	17:51 ± 01:47	**4.780 ± 1.530**	49.685 ± 3.239	07:06 ± 01:15	1.396 ± 2.161	2.040 ± 4.580	**-10:44 ± 02:11**
PC aa C42:2	**0.022 ± 0.007**	0.163 ± 0.013	23:54 ± 01:11	**0.024 ± 0.007**	0.166 ± 0.013	13:27 ± 01:06	0.002 ± 0.010	0.003 ± 0.018	**-10:27 ± 01:37**
PC ae C40:3	0.038 ± 0.028	0.873 ± 0.051	17:16 ± 02:53	0.042 ± 0.029	0.939 ± 0.051	23:06 ± 02:32	0.004 ± 0.041	0.067 ± 0.071	05:49 ± 03:50
SM C24:1	4.553 ± 4.120	105.360 ± 8.341	13:40 ± 03:23	6.606 ± 4.131	121.096 ± 8.337	00:55 ± 02:18	2.053 ± 5.835	15.736 ± 11.794	**11:14 ± 04:06**
Cit	0.676 ± 0.885	25.951 ± 1.634	19:11 ± 05:09	**1.874 ± 0.911**	28.562 ± 1.634	01:40 ± 01:48	1.198 ± 1.270	2.610 ± 2.311	06:28 ± 05:28
PC aa C42:5	0.006 ± 0.007	0.182 ± 0.014	15:21 ± 04:43	0.009 ± 0.007	0.210 ± 0.014	01:54 ± 03:01	0.003 ± 0.010	0.028 ± 0.019	10:32 ± 05:36
PC ae C44:4	0.010 ± 0.006	0.157 ± 0.013	17:39 ± 02:17	0.009 ± 0.006	0.191 ± 0.013	00:30 ± 02:32	-0.001 ± 0.009	0.034 ± 0.018	06:51 ± 03:25
PC aa C40:3	0.002 ± 0.012	0.328 ± 0.024	03:35 ± 05:15	0.009 ± 0.011	0.339 ± 0.024	16:58 ± 05:00	0.007 ± 0.016	0.011 ± 0.034	-10:37 ± 05:40
PC ae C42:4	0.021 ± 0.019	0.513 ± 0.027	15:44 ± 03:29	0.026 ± 0.020	0.571 ± 0.027	23:19 ± 02:48	0.004 ± 0.027	0.058 ± 0.039	07:35 ± 04:28
Ala	**35.568 ± 14.124**	372.478 ± 22.718	10:45 ± 01:28	11.676 ± 13.707	389.346 ± 22.718	19:21 ± 04:37	-23.892 ± 19.682	16.868 ± 32.128	08:35 ± 04:50
C18:1	**0.012 ± 0.003**	0.064 ± 0.002	20:58 ± 00:51	**0.006 ± 0.003**	0.075 ± 0.002	11:34 ± 01:39	-0.006 ± 0.004	*0.010 ± 0.003*	**-09:23 ± 01:52**
Taurine	**18.702 ± 4.581**	88.146 ± 5.696	10:56 ± 00:54	**16.592 ± 4.520**	88.797 ± 5.696	09:16 ± 01:02	-2.110 ± 6.435	0.650 ± 8.055	-01:39 ± 01:22
PC aa C40:2	**0.054 ± 0.012**	0.262 ± 0.021	00:14 ± 00:49	**0.066 ± 0.012**	0.211 ± 0.021	12:50 ± 00:39	0.012 ± 0.017	-0.051 ± 0.030	**-11:24 ± 01:03**
ADMA	0.003 ± 0.019	0.503 ± 0.020	14:51 ± 03:54	0.005 ± 0.019	0.485 ± 0.020	03:22 ± 14:20	0.002 ± 0.027	-0.018 ± 0.028	-11:28 ± 07:22
Serotonin	**0.277 ± 0.055**	0.538 ± 0.058	10:40 ± 00:44	**0.175 ± 0.054**	0.580 ± 0.058	08:46 ± 01:11	-0.102 ± 0.078	0.042 ± 0.082	-01:54 ± 01:24
Glu	3.478 ± 2.733	45.856 ± 3.217	06:41 ± 03:06	**12.916 ± 2.756**	57.897 ± 3.217	04:04 ± 00:49	**9.437 ± 3.881**	*12.041 ± 4.549*	-02:37 ± 03:13
PC aa C24:0	**0.008 ± 0.004**	0.052 ± 0.013	18:10 ± 01:41	0.006 ± 0.004	0.074 ± 0.013	23:43 ± 02:15	-0.002 ± 0.005	0.022 ± 0.019	05:33 ± 02:49

Table S2. Factor loadings from PCA.

Metabolite	*Cluster 1*	*Cluster 2*	*Cluster 3*
PC aa C30:0	0.8518	0.1871	0.2281
PC ae C34:3	0.8342	0.3633	0.1602
PC aa C36:6	0.7950	0.0338	0.4794
PC aa C32:1	0.7771	0.1239	0.2063
PC ae C34:0	0.7743	0.3940	0.1596
PC ae C32:1	0.7708	0.2725	0.3604
PC ae C34:2	0.7694	0.4099	0.3130
PC ae C34:1	0.7619	0.4193	0.4420
PC aa C34:3	0.7608	0.3060	0.3267
PC aa C34:4	0.7437	0.4689	0.0047
PC aa C32:0	0.7379	0.2883	0.4098
PC ae C36:2	0.7331	0.4612	0.3077
PC ae C38:6	0.7312	0.3603	0.4370
PC ae C36:5	0.7272	0.5836	0.0472
PC ae C36:1	0.7160	0.5763	0.2829
PC ae C36:0	0.7129	0.3017	0.2942
PC ae C32:2	0.7086	0.2015	0.4913
PC aa C32:3	0.7044	0.4713	0.2549
SM C18:0	0.7040	0.3772	0.4558
PC ae C36:3	0.7007	0.4249	0.4666
PC aa C34:1	0.6952	0.4040	0.2757
SM C18:1	0.6876	0.4463	0.3568
PC aa C40:6	0.6852	0.1248	0.5070
PC aa C38:6	0.6771	0.0591	0.6163
PC ae C30:0	0.6765	0.4455	0.1348
SM C16:0	0.6719	0.3283	0.5753
PC ae C38:0	0.6568	0.0768	0.6182
PC aa C38:0	0.6536	0.0670	0.6482
PC ae C42:2	0.6526	0.3882	0.4630
SM (OH) C22:1	0.6415	0.4816	0.4380
SM (OH) C16:1	0.6407	0.4038	0.4867
SM (OH) C22:2	0.6398	0.4895	0.4906
PC aa C36:0	0.6364	-0.1855	0.1928
SM C16:1	0.6325	0.4272	0.5032
PC ae C40:1	0.6224	0.2105	0.4994
SM (OH) C14:1	0.6059	0.4065	0.4829
PC aa C36:1	0.6038	0.4676	0.2980
PC aa C36:5	0.6006	0.4545	0.1752
Tyr	0.5937	0.5115	0.4079
PC aa C36:3	0.5929	0.5389	0.3012
PC aa C36:4	0.5864	0.5849	0.1162
PC ae C40:6	0.5835	0.3392	0.5614
PC aa C36:2	0.5752	0.1542	0.0777
PC aa C42:6	0.5705	0.4315	0.4773
PC aa C28:1	0.5585	0.5120	0.3395
SM C24:0	0.5489	0.4493	0.4599
PC aa C38:3	0.5359	0.5314	0.1815
lysoPC a C28:1	0.5358	0.3585	0.2409
PC aa C34:2	0.5352	0.0475	0.0478
SM C20:2	0.5285	0.4055	0.4675
PC ae C42:1	0.5183	0.4020	0.3981
Lys	0.5147	0.5114	0.1668
PC ae C38:2	0.5067	0.2276	0.1580
PC ae C30:2	0.4774	0.4564	0.3445
C9	0.4332	0.1441	–0.0103
C3-DC (C4-OH)	0.3010	0.0349	–0.0235
C14:2	0.2665	0.1879	0.1665
lysoPC a C20:4	–0.0101	0.8838	0.0602
lysoPC a C17:0	0.2864	0.8296	0.2469
PC ae C38:4	0.3753	0.8175	0.1354
C0	0.3445	0.8027	0.0215
Leu	0.2036	0.7949	0.1873
Ile	0.2252	0.7934	0.2380
lysoPC a C20:3	0.1669	0.7794	0.1466
PC ae C40:4	0.3375	0.7766	0.3024
PC aa C38:4	0.4443	0.7762	0.0559

Metabolite	*Cluster 1*	*Cluster 2*	*Cluster 3*
Val	0.2820	0.7706	0.2088
lysoPC a C16:0	0.3401	0.7566	0.3287
PC ae C38:5	0.4991	0.7449	0.3058
lysoPC a C18:0	0.3910	0.7336	0.2591
PC aa C38:5	0.4294	0.7327	0.1785
Phe	0.3547	0.7179	0.3995
PC ae C36:4	0.5722	0.7163	0.2003
PC ae C40:5	0.4230	0.7104	0.3644
Trp	0.4416	0.7099	0.3124
Kynurenine	0.3481	0.7064	0.2498
His	0.2715	0.7023	0.5296
lysoPC a C18:1	0.1288	0.6988	0.3888
PC aa C40:4	0.5389	0.6968	–0.0272
C18:2	0.1301	0.6828	0.2898
PC ae C44:6	0.0024	0.6476	0.4994
PC aa C40:5	0.5144	0.6450	0.1565
PC ae C42:5	0.1487	0.6444	0.5004
PC ae C38:3	0.5709	0.6400	0.2987
C2	–0.0428	0.6349	0.2986
Met	0.3304	0.6123	0.4065
lysoPC a C16:1	0.2532	0.6080	0.5139
PC ae C44:5	–0.0678	0.5912	0.5636
SDMA	0.0753	0.5766	0.4849
lysoPC a C18:2	0.1529	0.5692	0.3447
PC aa C42:4	0.4164	0.5664	0.3682
Sarcosine	0.2643	0.4963	0.2836
Pro	–0.0636	0.4846	0.2859
Creatinine	0.1462	0.4673	–0.0016
lysoPC a C24:0	0.2961	0.4662	0.2215
lysoPC a C28:0	0.2359	0.3732	–0.0828
C5-DC (C6-OH)	0.2712	0.3677	0.1028
C14:1	0.3511	0.3515	0.3229
lysoPC a C26:1	0.2674	0.3429	0.0106
C7-DC	0.1548	0.2932	0.0932
lysoPC a C26:0	0.0894	0.2112	–0.0397
Gly	0.1471	0.2560	0.8531
Asn	0.1228	0.2160	0.8246
PC aa C42:0	0.2630	0.2063	0.8094
Arg	0.1752	0.0219	0.7967
Thr	0.0952	0.2863	0.7950
PC aa C42:1	0.3999	0.2064	0.7844
Ser	0.2149	0.1890	0.7754
PC ae C40:2	0.5535	0.1970	0.7526
Gln	0.2074	0.3683	0.7501
PC ae C42:3	0.5508	0.2333	0.7270
SM C26:1	0.5956	0.0428	0.7198
PC ae C44:3	0.3615	0.3515	0.7115
SM C26:0	0.5380	0.1611	0.7081
SM (OH) C24:1	0.5193	0.2893	0.7012
Orn	0.0616	0.2104	0.6993
PC aa C42:2	0.3804	0.1259	0.6988
PC ae C40:3	0.5572	0.3702	0.6665
SM C24:1	0.5633	0.2955	0.6659
Cit	0.1145	0.4092	0.6645
PC aa C42:5	0.5529	0.3062	0.6595
PC ae C44:4	0.0064	0.4185	0.6519
PC aa C40:3	0.5093	0.3241	0.6490
PC ae C42:4	0.1840	0.5706	0.6283
Ala	0.4101	0.3311	0.5538
C18:1	0.0213	0.4960	0.5286
Taurine	0.0607	0.0395	0.4872
PC aa C40:2	0.3014	0.1923	0.4706
ADMA	0.1413	0.4010	0.4461
Serotonin	0.1649	-0.1272	0.3241
Glu	0.1356	0.2405	0.2728
PC aa C24:0	–0.2173	0.1859	–0.2533

Table S3. Pathway analysis for the simulated day shift schedule.

Pathway	Total	Expected	Hits	*p*	–log(*p*)	Holm *p*	FDR	Impact
Aminoacyl-tRNA biosynthesis	75	0.467	8	$3.30 \cdot 10^{-9}$	19.53	$2.64 \cdot 10^{-7}$	**$2.64 \cdot 10^{-7}$**	0.113
Nitrogen metabolism	39	0.243	5	$2.29 \cdot 10^{-6}$	12.99	$1.81 \cdot 10^{-4}$	**$9.16 \cdot 10^{-5}$**	0.008
Alanine, aspartate and glutamate metabolism	24	0.150	4	$9.66 \cdot 10^{-6}$	11.55	$7.53 \cdot 10^{-4}$	**$2.58 \cdot 10^{-4}$**	0.486
Arginine and proline metabolism	77	0.480	5	$6.87 \cdot 10^{-5}$	9.59	$5.29 \cdot 10^{-3}$	**$1.37 \cdot 10^{-3}$**	0.312
Cyanoamino acid metabolism	16	0.100	3	0.0001	9.17	$7.94 \cdot 10^{-3}$	**$1.67 \cdot 10^{-3}$**	0
D-Arginine and D-ornithine metabolism	8	0.050	2	0.0010	6.91	0.075	**0.013**	0
Glutathione metabolism	38	0.237	3	0.0015	6.54	0.107	**0.017**	0.011
D-glutamine and D-glutamate metabolism	11	0.069	2	0.0019	6.25	0.141	**0.019**	0.139
Glycine, serine and threonine metabolism	48	0.299	3	0.0029	5.86	0.206	**0.025**	0.420
Taurine and hypotaurine metabolism	20	0.125	2	0.0065	5.04	0.458	0.052	0.363
Sphingolipid metabolism	25	0.156	2	0.0100	4.60	0.701	0.073	0.010
Methane metabolism	34	0.212	2	0.0181	4.01	1.000	0.121	0.018
Porphyrin and chlorophyll metabolism	104	0.648	3	0.0244	3.71	1.000	0.150	0
Primary bile acid biosynthesis	47	0.293	2	0.0333	3.40	1.000	0.191	0.016
Cysteine and methionine metabolism	56	0.349	2	0.0460	3.08	1.000	0.245	0.012
Linoleic acid metabolism	15	0.093	1	0.0898	2.41	1.000	0.449	0
Sulfur metabolism	18	0.112	1	0.1068	2.24	1.000	0.489	0
Purine metabolism	92	0.573	2	0.1100	2.21	1.000	0.489	0
Selenoamino acid metabolism	22	0.137	1	0.1290	2.05	1.000	0.543	0
Thiamine metabolism	24	0.150	1	0.1399	1.97	1.000	0.560	0
Valine, leucine and isoleucine biosynthesis	27	0.168	1	0.1561	1.86	1.000	0.595	0
Alpha-linolenic acid metabolism	29	0.181	1	0.1667	1.79	1.000	0.606	0
Glycerophospholipid metabolism	39	0.243	1	0.2179	1.52	1.000	0.743	0.101
Butanoate metabolism	40	0.249	1	0.2228	1.50	1.000	0.743	0
Histidine metabolism	44	0.274	1	0.2424	1.42	1.000	0.776	0.001
Lysine degradation	47	0.293	1	0.2567	1.36	1.000	0.790	0
Pyrimidine metabolism	60	0.374	1	0.3160	1.15	1.000	0.928	0
Arachidonic acid metabolism	62	0.386	1	0.3247	1.12	1.000	0.928	0
Tryptophan metabolism	79	0.492	1	0.3947	0.93	1.000	1.000	0.057

Table S4. Pathway analysis for the simulated night shift schedule.

Pathway	**Total**	**Expected**	**Hits**	***p***	**–log(*p*)**	**Holm *p***	**FDR**	**Impact**
Aminoacyl-tRNA biosynthesis	75	0.561	10	$1.68 \cdot 10^{-11}$	24.81	$1.35 \cdot 10^{-9}$	$\mathbf{1.35 \cdot 10^{-9}}$	0.056
Nitrogen metabolism	39	0.292	4	0.0002	8.78	0.012	$\mathbf{4.85 \cdot 10^{-3}}$	0
Arginine and proline metabolism	77	0.576	5	0.0002	8.61	0.014	$\mathbf{4.85 \cdot 10^{-3}}$	0.365
Valine, leucine and isoleucine biosynthesis	27	0.202	3	0.0009	6.99	0.071	**0.018**	0.027
D-Arginine and D-ornithine metabolism	8	0.060	2	0.0014	6.54	0.109	**0.023**	0
Taurine and hypotaurine metabolism	20	0.150	2	0.0093	4.68	0.695	0.124	0.363
Alanine, aspartate and glutamate metabolism	24	0.179	2	0.0132	4.33	0.979	0.151	0.264
Glycerophospholipid metabolism	39	0.292	2	0.0332	3.40	1.000	0.310	0.104
Valine, leucine and isoleucine degradation	40	0.299	2	0.0348	3.36	1.000	0.310	0.022
Glycine, serine and threonine metabolism	48	0.359	2	0.0486	3.02	1.000	0.389	0.146
Biotin metabolism	11	0.082	1	0.0794	2.53	1.000	0.529	0
D-glutamine and D-glutamate metabolism	11	0.082	1	0.0794	2.53	1.000	0.529	0.027
Linoleic acid metabolism	15	0.112	1	0.1068	2.24	1.000	0.657	0
Tryptophan metabolism	79	0.591	2	0.1160	2.15	1.000	0.663	0.092
Selenoamino acid metabolism	22	0.165	1	0.1528	1.88	1.000	0.815	0
Sphingolipid metabolism	25	0.187	1	0.1719	1.76	1.000	0.828	0.010
Phenylalanine, tyrosine and tryptophan biosynthesis	27	0.202	1	0.1844	1.69	1.000	0.828	0.001
Beta-alanine metabolism	28	0.209	1	0.1905	1.66	1.000	0.828	0
Alpha-linolenic acid metabolism	29	0.217	1	0.1967	1.63	1.000	0.828	0
Lysine biosynthesis	32	0.239	1	0.2148	1.54	1.000	0.859	0.100
Glutathione metabolism	38	0.284	1	0.2498	1.39	1.000	0.952	0
Histidine metabolism	44	0.329	1	0.2834	1.26	1.000	0.959	0.140
Phenylalanine metabolism	45	0.337	1	0.2889	1.24	1.000	0.959	0.119
Primary bile acid biosynthesis	47	0.351	1	0.2997	1.21	1.000	0.959	0.008
Lysine degradation	47	0.351	1	0.2997	1.21	1.000	0.959	0.147
Cysteine and methionine metabolism	56	0.419	1	0.3464	1.06	1.000	1.000	0
Pyrimidine metabolism	60	0.449	1	0.3662	1.00	1.000	1.000	0
Arachidonic acid metabolism	62	0.464	1	0.3759	0.98	1.000	1.000	0
Purine metabolism	92	0.688	1	0.5054	0.68	1.000	1.000	0
Porphyrin and chlorophyll metabolism	104	0.778	1	0.5497	0.60	1.000	1.000	0

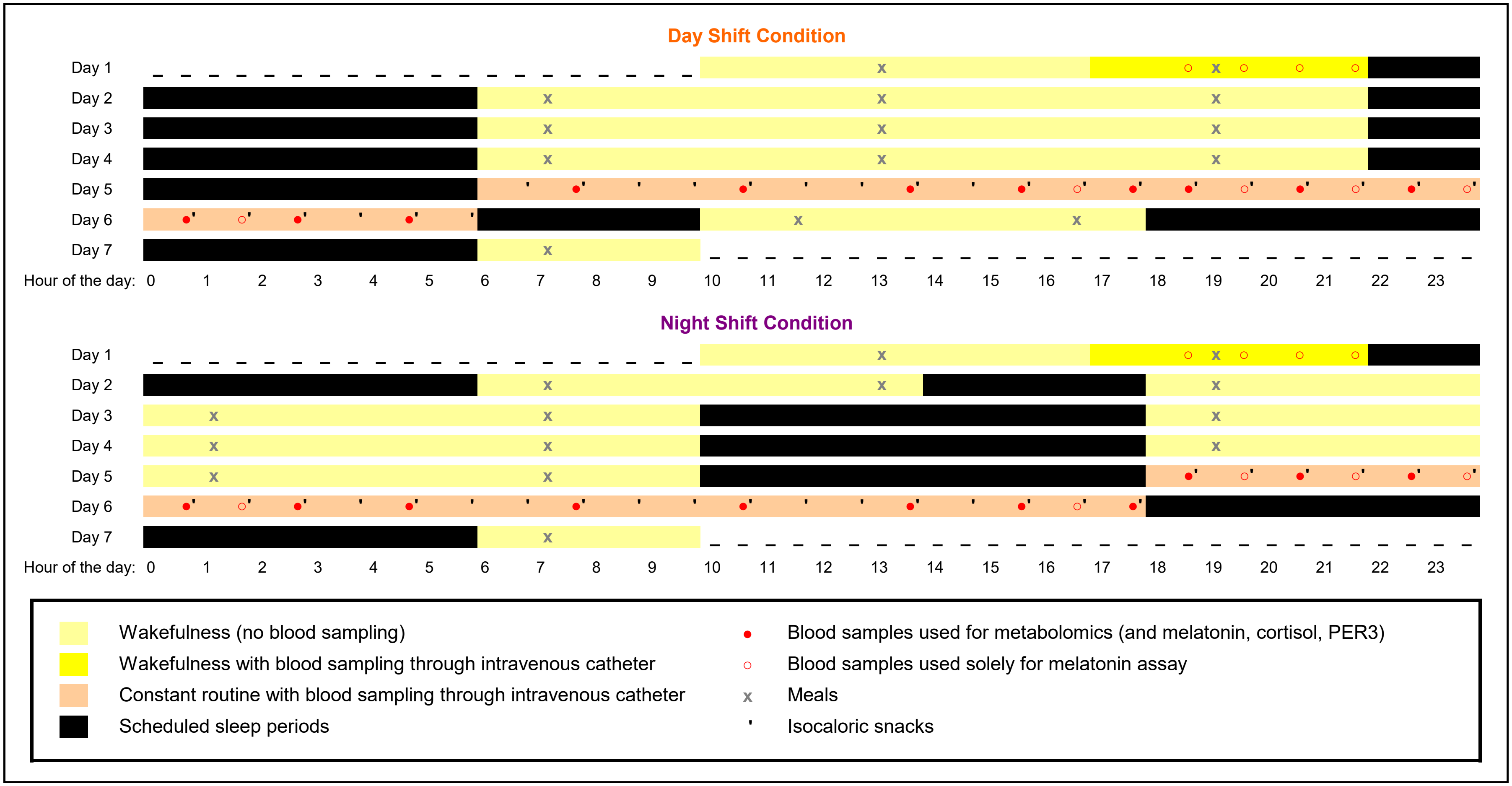

Fig. S1. In-laboratory study protocol comprising an adaptation period, 3 days on a simulated day shift or night shift schedule, 24 h under constant routine – sustained wakefulness in constant ambient temperature and dim light, fixed posture, and hourly, identical snacks (which were all consumed) – and a recovery period.

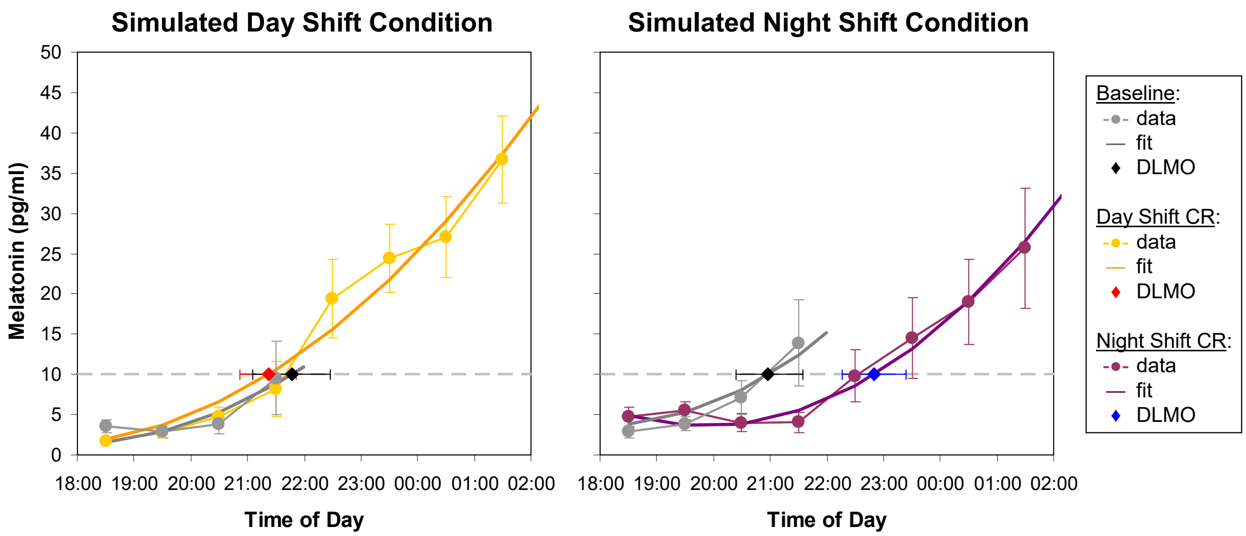

Fig. S2. Plasma melatonin and dim light melatonin onset (DLMO) at baseline and during the constant routine (CR) after simulated day shift work (left) and night shift work (right). For the day shift condition, the threshold of 10 pg/ml was crossed during baseline at 21:47 (± 41 min) and during the constant routine at 21:22 (± 30 min) – indicating a phase difference (advance) of –25 min (± 37 min), which was not statistically significant (t_6=–0.66, p=0.53). For the night shift condition, the threshold was crossed during baseline at 20:58 (± 37 min) and during the constant routine at 22:50 (± 34 min) – indicating a phase difference (delay) of 112 min (± 32 min), which was statistically significant (t_6=3.53, p=0.012). There was no significant difference in the baseline phase of the DLMO between the two groups (t_{12}=–1.23, p=0.24); but during the constant routine, the timing of the DLMO was significantly later by 88 min (± 32 min) in the night shift condition (t_{12}=2.72, p=0.019). Gray/black: baseline. Orange/red: constant routine after simulated day shift condition. Purple/blue: constant routine after simulated night shift condition. Light tinted circles: group means (± SE). Dark tinted curves: Taylor series approximation fits. Dashed line: 10 pg/ml threshold for DLMO. Diamonds: timing of DLMO (± SE).

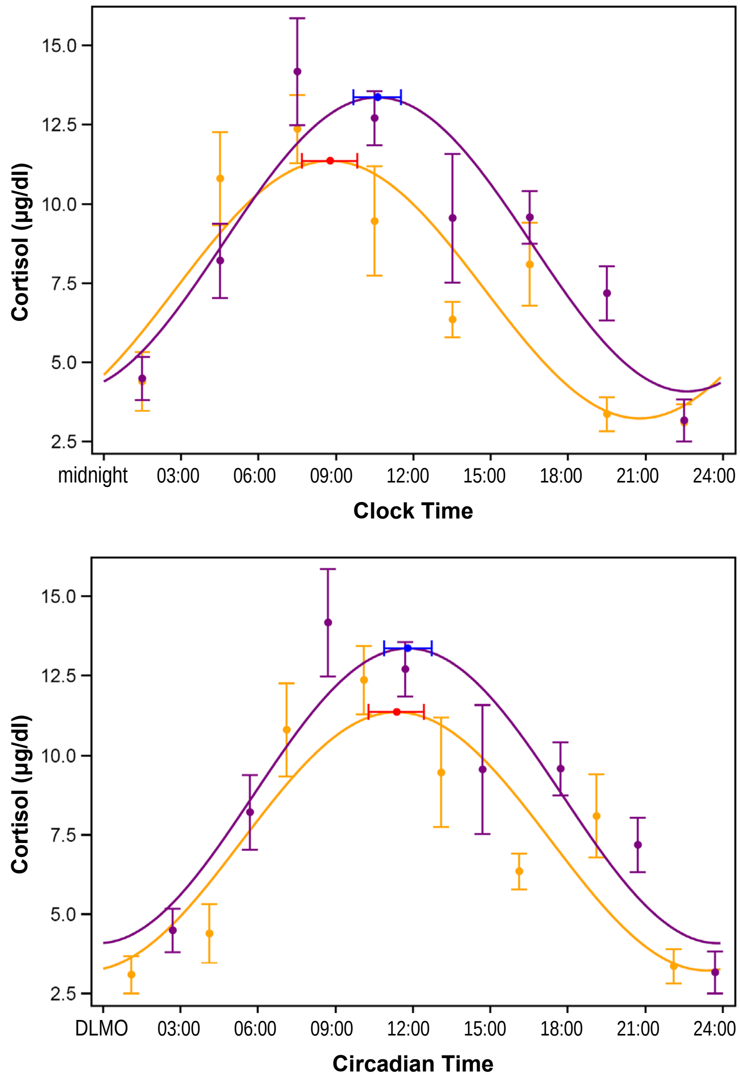

Fig. S3. Plasma cortisol during constant routine after simulated day shift work (orange) and night shift work (purple), plotted against time of day (top) and against circadian time relative to DLMO (bottom). For the day shift condition, there was a significant 24-h rhythm (t_6=7.07, p<0.001), and the acrophase occurred at 08:46 (± 32 min). For the night shift condition, there was also a significant 24-h rhythm (t_6=8.16, p<0.001), and the acrophase occurred at 10:36 (± 27 min). The phase difference between the two groups during the constant routine involved a delay of 110 min (± 42 min) in the night shift condition, which was statistically significant (t_{12}=2.60, p=0.023) and was similar to the delay of 88 min (± 32 min) seen for the DLMO (see Fig. S2). Orange/red: constant routine after simulated day shift condition. Purple/blue: constant routine after simulated night shift condition. Circles: group means (± SE). Curves: cosinor fits. Red and blue markers: timing of acrophase (and 95% confidence interval).

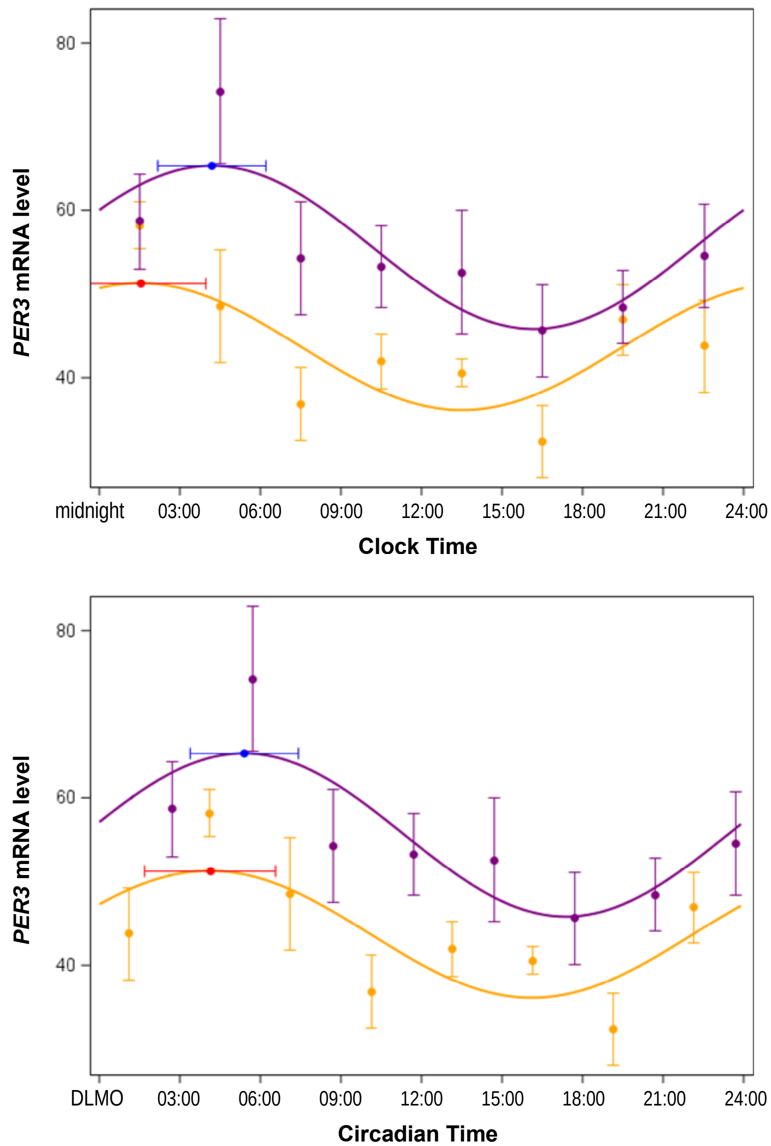

Fig. S4. Transcript levels of *PER3* gene expression during constant routine after simulated day shift work (orange) and night shift work (purple), plotted against time of day (top) and against circadian time relative to DLMO (bottom). For the day shift condition, there was a significant 24-h rhythm (t_6=3.14, p=0.010), and the acrophase occurred at 01:30 (± 73 min). For the night shift condition, there was also a significant 24-h rhythm (t_6=3.93, p=0.004), and the acrophase occurred at 04:11 (± 60 min). The phase difference between the two groups during the constant routine involved a delay of 160 min (± 76 min) in the night shift condition, which was not statistically significant (t_{12}=1.70, p=0.12) but was similar to the delay of 100 min (± 67 min) in the circadian rhythm of plasma cortisol (see Fig. S3). Orange/red: constant routine after simulated day shift condition. Purple/blue: constant routine after simulated night shift condition. Circles: group means (± SE). Curves: cosinor fits. Red and blue markers: timing of acrophase (and 95% confidence interval).

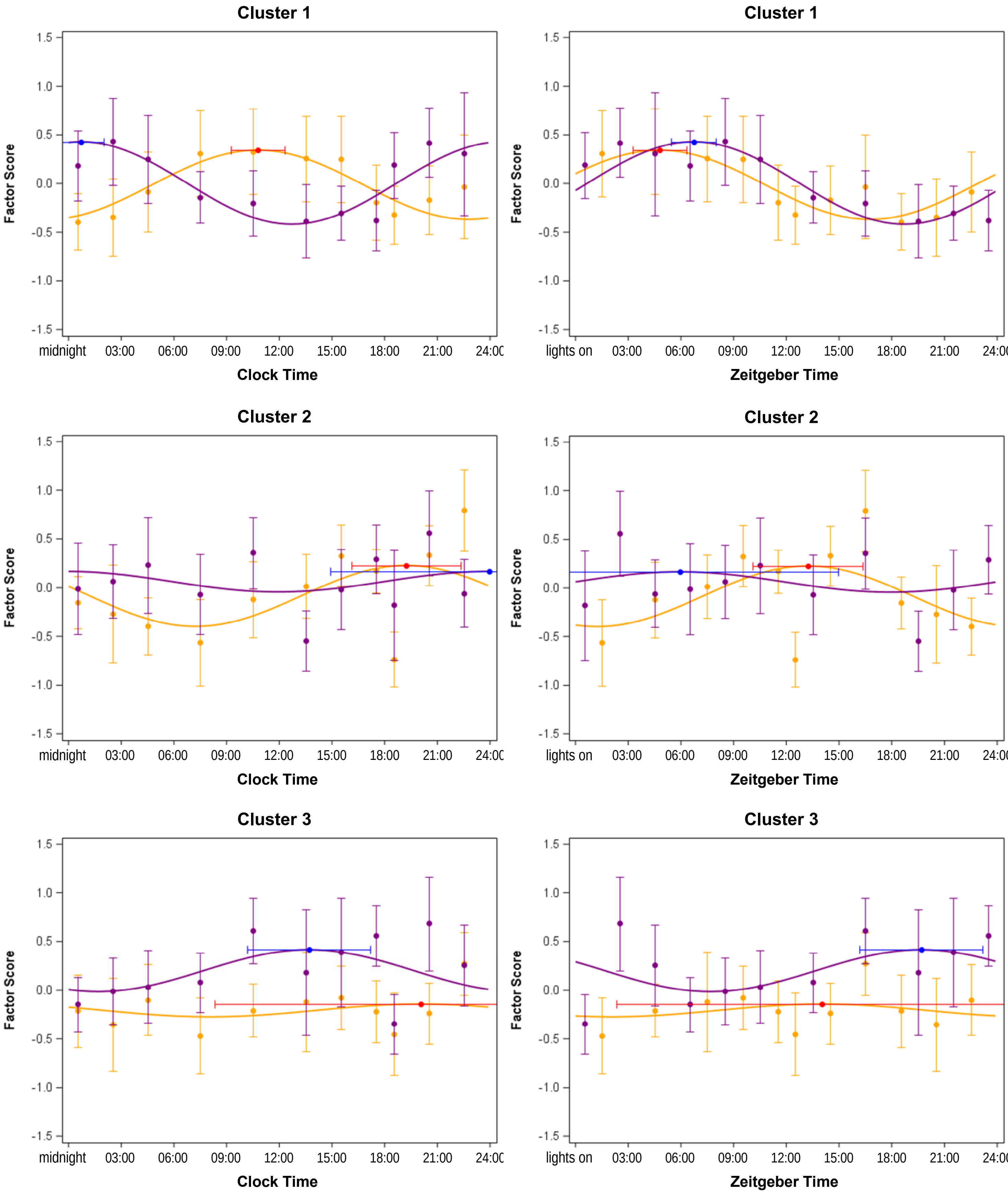

Fig. S5. Temporal profiles of the three principal component clusters of the metabolites measured during constant routine, plotted against clock time (time of day, left) and against zeitgeber time (time in constant routine since scheduled awakening, right). Orange/red: constant routine after simulated day shift schedule. Purple/blue: constant routine after simulated night shift schedule. Circles: group means (± SE). Curves: cosinor fits. Red and blue markers: timing of acrophase (and 95% confidence interval).

Fig. S6. Heat maps of metabolites during the 24-h constant routine, plotted as Z scores (standard deviations away from the mean) against clock time in 6-h blocks. The dominant response to the experiment was a complete reversal of 24-h rhythmicity following the simulated night shift schedule. Red/green continuum indicates Z score (see scale on top). Order of metabolites is based on factor loadings from PCA (Table S2). D = day shift condition; N = night shift condition. 03 = 00:00–06:00; 09 = 06:00–12:00; 15 = 12:00–18:00; 21 = 18:00–24:00.

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