SUPPORTING INFORMATION to:

Lipidomics reveals diurnal lipid oscillations in human skeletal muscle persisting in cellular myotubes cultured *in vitro*

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SI Materials and Methods

Human skeletal muscle biopsies for the in vivo study

The volunteers of the *in vivo* study had neither history of sleep disorder as confirmed by pre-study questionnaires MCTQ (1), Horne-Ostberg (2) and PSQI (3) (see Tables S4-S6), nor did they participate in a shifted work schedule, or travelled across more than two time zones within three weeks preceding the study. Each participant adhered to a consistent daily feeding and sleeping routine. Specifically, participants woke between 06:00 and 07:00 h and went to bed between 22:00 and 23:00 h (with individual wake-up and sleep times recorded during the week and verified via time-stamped voicemails) - resulting in an average of 8 h sleep opportunity. In addition, participants exposed themselves to 15 min natural light within 1.5 h of waking, as verified by wearable light sensors (ActiWatch™). Self-selected meals were scheduled at 08:00 h, 12:00 h and 18:00 h, with defined snacks at 10:00 h, 15:00 h and 20:00 h daily. Participants arrived in the laboratory at 19:00 h on the day prior to the testing day and ingested a standardized meal and snack (6405 kJ, 132 g carbohydrate, 76 g fat, 70 g protein) that first evening and remained for the duration of their stay in a semi-recumbent position. The resting metabolic rate was measured via indirect calorimetry the next morning according to best practice and used to calculate individual energy requirements at rest, which were then precisely met on an individual basis by prescribing hourly ingestion during waking hours of a mixed-macronutrient meal-replacement solution (Resource, Nestlé, CH) containing 5270 kJ/L, 140 g/L carbohydrate, 35 g/L fat, 94 g/L protein in small volumes relative to metabolic requirements (i.e. 92 ± 17 ml) to ensure energy and nutrient balance. The laboratory was free from natural light and with artificial lighting standardized to 800 lux in the direction of gaze, ambient temperature maintained between 20-25°C (23.6 ± 3.62°C; mean ± SD) and noise levels tightly regulated. Participants were not permitted to sleep during waking hours when

lights were on (07:00-22:00 h) and wore eye masks whilst trying to sleep during lights-out (22:00-07:00 h). Participants remained asleep during hourly blood sampling throughout the night but were woken briefly for acquisition of the nighttime muscle biopsy samples but without removing their eye masks. Anesthetic administration (1% lidocaine w/o epinephrine) and skin/fascia incisions for this procedure (4) were completed within the hour prior to sleep such that night-time samples could be acquired within several minutes from pre-prepared sites with minimal discomfort. Three samples were taken from each leg in alternating and ascending order (starting with the non/dominant limb counterbalanced between participants), with skin incisions separated by 2-3 cm.

Melatonin and cortisol analyses

Blood samples were drawn every hour, starting on the testing day throughout both the waking and sleeping periods. Samples were obtained using an intravenous cannula fitted to the antecubital vein. The first 2 ml was drawn and discarded as waste before a further 10 ml was extracted. Of this 10 ml, 2 ml was dispensed in to a centrifuge tube containing lithium heparin, 4 ml was allocated to a plain serum tube and the remaining 4 ml was deposited in a tube coated with EDTA. The lithium heparin and EDTA tubes were both immediately centrifuged for 10 minutes (3466 g, 4°C) after which the supernatants were removed and stored at -80°C. Melatonin was measured as previously described (5) and cortisol was measured by COBAS (Roche).

In vitro skeletal myotube synchronization and real-time bioluminescence recording

Myoblasts were transduced with a *Bmal1* luciferase (*Bmal1-luc*) (6) containing lentiviral reporter with a multiplicity of infection (MOI) = 3, grown to confluence, and subsequently differentiated into myotubes, as previously described by us in (7). For lentiviral production

see (8). To synchronize primary myotubes, 10 µM forskolin (Sigma) was added to the culture medium for 1 h, followed by a medium change. For bioluminescence recording the culture medium was changed to the phenol red-free recording medium containing 100 µM luciferin (D-luciferin 306-250, NanoLight Technology) and cells were transferred to a 37°C light-tight incubator (Prolume LTD), as previously described by us (8). Bioluminescence from each dish was continuously monitored using a Hamamatsu photomultiplier tube (PMT) detector assembly. Photon counts were integrated over 1 min intervals. Bioluminescence profiles are shown as detrended data. For detrended time series, luminescence signals were smoothened by a moving average with a window of 24 h. For this, each data point was divided by the average of data points in an interval of 24 h (12 h before and 12 h after the analyzed data point).

Materials for lipid extraction

Synthetic lipid standards [PC 12:0/12:0 (850335), PE 17:0/14:1 (LM-1104), PI 17:0/14:1 (LM-1504), PS 17:0/14:1 (LM-1304), Cer d18:1/17:0 (860517), SM d18:1/12:0 (860583), GlcCer d18:1/8:0 (860540)] were from Avanti Polar Lipids Inc. MTBE and methylamine (33% in absolute ethanol) were purchased from Sigma. Chloroform, methanol, n-butanol and ammonium molybdate were from Acros Organics. LC-MS grade methanol, water and ammonium acetate were from Fluka. HPLC grade chloroform was purchased from Acros Organics. Monopotassium phosphate and L-ascorbic acid were from Sigma. 70% perchloric acid was from Merck.

Lipid extraction procedure

Lipid extracts were prepared using the MTBE protocol (9). Briefly, ~30 mg ground skeletal muscle tissue or human primary myotubes harvested from one confluent 10 cm dish (~1.5 x

10^6 cells) were resuspended in 100 µl H_2O. 360 µl methanol and a mix of internal standards were added (400 pmol PC 12:0/12:0, 1000 pmol PE 17:0/14:1, 1000 pmol PI 17:0/14:1, 3300 pmol PS 17:0/14:1, 2500 pmol SM d18:1/12:0, 500 pmol Cer d18:1/17:0 and 100 pmol GlcCer d18:1/8:0). After addition of 1.2 ml of MTBE, samples were placed for 10 min on a multitube vortexer at 4°C followed by incubation for 1 h at room temperature (RT) on a shaker. Phase separation was induced by addition of 200 µl MS-grade water. After 10 min at RT samples were centrifuged at 1000 g for 10 min. The upper (organic) phase was transferred into a 13 mm glass tube and the lower phase was re-extracted with 400 µl artificial upper phase [MTBE/methanol/H_2O (10:3:1.5, v/v/v)]. The combined organic phases were dried in a vacuum concentrator (CentriVap, Labconco). Lipids were dissolved in chloroform/methanol and divided into three aliquots. One aliquot was treated by alkaline hydrolysis to enrich for sphingolipids and the other two aliquots were used for glycerophospholipid and phosphorus assay, respectively. Glycerophospholipids were deacylated according to the method by Clarke (10). Briefly, 1 ml freshly prepared monomethylamine reagent [methylamine/H_2O/n-butanol/methanol (5:3:1:4, (v/v/v/v)] was added to the dried lipid extract and then incubated at 53°C for 1 h in a water bath. Lipids were cooled to RT and then dried. For desalting, the dried lipid extract was resuspended in 300 µl water-saturated n-butanol and then extracted with 150 µl H_2O. The organic phase was collected, and the aqueous phase was re-extracted twice with 300 µl water-saturated n-butanol. The organic phases were pooled and dried in a vacuum concentrator.

Determination of total phosphorus

100 µl of the total lipid extract, resuspended in chloroform/methanol (1:1), were placed into 13 mm disposable pyrex tubes and dried in a vacuum concentrator. 0, 2, 5, 10, 20 µl of a 3 mM KH_2PO_4 standard solution were placed into separate pyrex tubes. To each tube 20 µl of

water and 140 µl of 70% perchloric acid were added. Samples were heated at 180°C for 1 h in a chemical hood. Then, 800 µl of a freshly prepared solution of water, ammonium molybdate (100 mg/8 ml H_2O) and ascorbic acid (100 mg/6 ml H_2O) in a ratio of 5:2:1 (v/v/v) were added. Tubes were heated at 100°C for 5 min with a marble on each tube to prevent evaporation. Tubes were cooled at RT for 5 min. 100 µl of each sample was then transferred into a 96-well microplate and the absorbance at 820 nm was measured.

Phospho- and sphingolipid analysis by mass spectrometry

Mass spectrometry analysis for the identification and quantification of phospho- and sphingolipid species was performed on a TSQ Vantage Triple Stage Quadrupole Mass Spectrometer (Thermo Fisher Scientific) equipped with a robotic nanoflow ion source (Nanomate HD, Advion Biosciences), using multiple reaction monitoring (MRM). Each individual ion dissociation pathway was optimized with regard to collision energy. Dried lipid extracts were resuspended in 250 µl MS grade chloroform/methanol (1:1) and further diluted in either chloroform/methanol (1:2) plus 5 mM ammonium acetate (negative ion mode) or in chloroform/methanol/H_2O (2:7:1) plus 5 mM ammonium acetate (positive ion mode). Lipid concentrations were calculated relative to the relevant internal standards and then normalized to the total phosphate content of each total lipid extract.

Triacylglyceride analysis

Mass spectrometry analysis for triacylglycerides (TAG) was performed on a hybrid ion trap LTQ-Orbitrap XL mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) equipped with a micro LC binary pump UFLC-XR (Shimadzu). The equivalent of 0.4 nmol of total phosphate content in 20mM ammonium hydroxide was injected onto a 2.6 µm, 50 x 2.1 mm Kinetex Hilic column (Phenomenex). Lipid extracts (2 µL injection volume) were

separated over an 8 minute gradient at a flow rate of 200 µL/min. Mobile phase composition and gradient were as follow: 0 min, 5% A; 1.7 min, 12%A, 3.5 min, 25% A and 4.5 min, 0% A where A was deionized water containing 10 mM ammonium formate and 0.5% formic acid and B was acetonitrile/methanol 10:1 (v/v) containing 10 mM ammonium formate and 0.5% formic acid. TAG ions were analyzed in positive mode in full scan experiment (m/z 200-2000) and detected as ammonium adducts $[M+NH4]^+$. MS survey scans were acquired with a resolution set at 60'000 (FWHM at m/z = 400) with an AGC set at 5.0E5, one microscan and maximum injection time set at 250 ms. The heated electrospray source HESI II was operated at a temperature of 100°C and a source voltage at 4.0KV. Sheath, auxiliary and sweep nitrogen gas arbitrary units were set at 15, 5, 1 respectively, while the transfer capillary temperature was set to 275°C. Data analysis: Mass spectrometry data were acquired with the LTQ Tuneplus 2.5 and treated with Xcalibur 2.1 (Thermo Fisher Scientific). Lipid identification was carried out with the Lipid Data Analyzer II (LDA v. 2.5.1, IGB-TUG Graz University) (11). Care was taken to calibrate the instrument regularly to ensure a mass accuracy consistently lower than 3 ppm thereby leaving only few theoretical possibilities for elemental assignment.

Data analysis

The clustering of the circadian lipid data into two groups was computed with a custom made procedure in MATLAB as follows. Step 1: the z scores were computed from raw lipid concentration data for each lipid/subject and after removal of outliers. Step 2: for each subject/lipid/biological replicate, the z-scores were detrended with the mean over 6 (*in vivo*) or 7 (*in vitro*) time points. Step 3: for each subject/lipid, the phase and amplitude for period 22 h (*in vitro*) and 24 h (*in vivo*) of a sinusoidal fit was computed using ordinary least squares using the two biological replicates per time point. P values of the coefficients were computed

assuming normality of the data. Step 4: for clustering, the signals were retained if they had a *p* value of the fit with FDR less than 0.05 (*in vitro*) or 0.10 (*in vivo*) using the Benjamini-Hochberg procedure applied over all lipids and subjects. The data were then clustered into two groups using k means in MATLAB (using 20 replicates). Step 5: two different sinusoids with fixed periods 22 h (*in vitro*) and 24 h (*in vivo*) were fit using ordinary least squares through all the data respecting the earlier determined clustering. The clustering was encoded with a standard 0/1 categorical variable. P values on the coefficients of the fit were calculated assuming normality of the data and verified to be significant (≤ 0.05) for each fitted sinusoid. Step 6: for visualizing the 95% confidence intervals of the fit, the standard errors on the coefficients were used in a Monte Carlo procedure to generate 500 fits.

Time-series for lipid data were analyzed by ARSER (12) through meta2d in MetaCycle R package (13). Period length was set between 20 and 28 h. Distribution plots were made by kernel smoothing as implemented by ksdensity in MATLAB with standard options. Boxplots were made with MATLAB (standard options) on the raw lipid concentration data (7 time points per lipid). Amplitude was calculated as difference of max and mix over the 7 time points.

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FIGURE LEGENDS

Figure S1. Representative PC metabolite profiles of phosphate normalized and unnormalized data *in vivo*. PC metabolite profiles for donor VII and donor X are shown. Lipid values were either not normalized to phosphate (upper panel) or normalized to total phosphate (lower panel). Normalized and unnormalized temporal lipid values were divided by each lipid mean value. Only lipids that were detected at all time points are shown.

Figure S2. Logarithmic scale heatmap of lipid abundance shown for phosphatidylserine (PS) and cardiolipin (CL). CL72:8_C18:2 is not detected *in vitro* (left column) but is highly abundant *in vivo* (right column). Colors show the log concentration for each metabolite. PS nomenclature: the total number of acyl carbons and double bonds is indicated. CL nomenclature: total number of acyl carbons in the four fatty acyl chains with presence of at least one fatty acid with a carbon number as indicated by underscore.

Figure S3. Representative PC metabolite profiles of phosphate normalized and unnormalized data *in vitro*. PC metabolite profiles for myotube cultures established from donor #5 and donor #9 are shown. Lipid values were either not normalized to phosphate (upper panel) or normalized to total phosphate (lower panel). Each lipid value (normalized or unnormalized) was divided by its lipid mean value.

Figure S4. Around-the-clock profile of the most abundant PC lipids in primary myotubes. The most abundant PC lipids exhibit enrichment 16 h and 28 h after synchronization.

Figure S5. Temporal lipid profile of PC32:2 in human skeletal myotubes. (A) The total profile of PC32:2 is not rhythmic in human primary myotubes (left panel). Individual lipid profiles are displaying oscillations albeit with different phase (middle panel). Lipid oscillations can be clustered into two major subgroups that reach their zenith 16 h or 24-28 h after synchronization, respectively (right panel). **(B)** TAG58:9 is not rhythmic in skeletal muscle across all subjects but displays subgroup oscillations upon individual analysis.

Figure S6. Lipid chain length but not the degree of desaturation influences the circadian profile of PC and SM lipid metabolites. (A) Temporal profile of PC, SM, and PE lipids, sorted by chain length in human primary myotubes. PC/SM lipids: only a subgroup of donors is shown that peak 28 h after forskolin synchronization (n=4). For PE, the average profile across all ten subjects is shown (n=10). PC, PE: the number represents the sum of carbon atoms in both fatty acyl chains; SM lipids: the number represents the sum of carbon atoms in the sphingoid base (C18) plus fatty acyl chain. **(B)** RT-qPCR results for *ACADL, ACADVL* and *ACSL3* on mRNA samples extracted from human skeletal muscle biopsies. Data were normalized to *GAPDH* (mean ± SEM). **(C)** PC lipid profiles (primary myotubes) sorted by their degree of desaturation. The number of double bonds is indicated.

Figure S7. Myosin isoform analysis. (A) RT-qPCR results for *MYH1, MYH2, MYH4* and *MYH7* on mRNA samples extracted from human skeletal muscle biopsies derived from two different origins (*vastus lateralis* or *gluteus maximus*). For donor characteristics see Table S1 and S2. Data were normalized to *GAPDH* (mean ± SEM). **(B)** Myosin isoform expression levels relative to *MYH1*.

Supplementary Tables

Table S1. Donor characteristics *vastus lateralis* (*in vivo* study)

Subject	Sex	Age (years)	BMI (kg/m^2)	Muscle biopsy
I	M	22	28.3	*vastus lateralis*
II	F	37	28.3	*vastus lateralis*
III	M	27	20.4	*vastus lateralis*
IV	M	33	23.6	*vastus lateralis*
V	M	54	25.6	*vastus lateralis*
VI	M	24	24.6	*vastus lateralis*
VII	M	25	23.8	*vastus lateralis*
VIII	M	30	20.3	*vastus lateralis*
IX	M	22	23.2	*vastus lateralis*
X	M	25	23.4	*vastus lateralis*
AVERAGE	**9/1**	**29.9 ± 9.8**	**24.1 ± 2.7**	***vastus lateralis***

Table S2. Donor characteristics *gluteus maximus* (*in vitro* study)

Subject	Sex	Age (years)	BMI (kg/m^2)	Fasting blood glucose (mmol/L)	Muscle biopsy
1	F	66	24.77	5.0	*gluteus maximus*
2	M	62	24.30	5.44	*gluteus maximus*
3*,#	F	77	25.56	5.98	*gluteus maximus*
4*	M	65	23.41	6.9	*gluteus maximus*
5*	F	65	25.85	5.06	*gluteus maximus*
6*	M	58	23.88	6.0	*gluteus maximus*
7*	M	59	24.62	4.88	*gluteus maximus*
8*	F	65	21.99	6.2	*gluteus maximus*
9#	F	65	22.77	4.95	*gluteus maximus*
10	M	48	24.30	5.5	*gluteus maximus*
AVERAGE 5/5		**63 ± 7.36**	**24.15 ± 1.19**	**5.59 ± 0.66**	***gluteus maximus***
11*,#	F	53	22.41	5.1	*gluteus maximus*
12*,#	M	57	26.3	6.2	*gluteus maximus*
13*	M	65	22.53	5.6	*gluteus maximus*
14*	M	64	22.04	5.4	*gluteus maximus*

Myotube cells of donors 1-10 were used for the *in vitro* lipidomic analysis.
* cDNA of these donor biopsies was used for myosin isoform analysis.
# Donors used for siRNA-mediated clock disruption experiment.

Table S3. Most significantly rhythmic lipids in human skeletal muscle and primary myotubes

Ion	Rhythmic *in vitro*	Ion	Rhythmic *in vivo*
TAG54:6	8/9	GlcCer42:2	5/7
GlcCer34:1	**7/10**	**SM38:1**	**5/7**
TAG50:4	6/10	SM42:2	5/7
TAG52:2	6/9	LysoPI	5/7
TAG54:3	6/9	PC(O)36:4	5/7
TAG56:7	6/9	PC(O)36:6	5/7
TAG56:8	6/9	PC32:2	5/7
TAG58:7	6/9	PC34:4	5/7
DHCer32:0	6/10	**GlcCer34:1**	**4/7**
PE30:1	6/10	GlcDHCer34:1	4/7
PE34:4	**6/10**	PC(O)32:0	4/7
SM32:1	6/10	PC(O)32:6	4/7
Cer42:1	5/10	PC(O)34:4	4/7
Cer36:1	5/10	PC(O)36:2	4/7
GlcCer34:1-OH	5/10	PC(O)36:3	4/7
GlcCer40:1	5/10	PC(O)36:5	4/7
GlcCer40:1-OH	5/10	PC30:0	4/7
GlcCer42:1	5/10	PC32:1	4/7
GlcDHCer40:0	5/10	PC34:2	4/7
PC42:1	5/10	PC36:3	4/7
PC42:3	5/10	PC36:5	4/7
PC42:6	5/10	PE(O)34:4	4/7
PC44:4	5/10	**PE34:4**	**4/7**
PE(O)34:1	5/10	PE36:0	4/7
PE(O)44:1	5/10	PE38:1	4/7
PE34:0	5/10	PI42:1	4/7
SM34:1	5/10	PI42:2	4/7
SM38:1	**5/10**	PS40:6	4/7
SM42:1	5/10	SM40:2	4/7
SM44:3	5/10	TAG44:6	4/7

Table S4. Munich Chronotype Questionaire

Munich Chronotype Questionnaire

Statement	Participant									
	1	2	3	4	5	6	7	8	9	10
On Workdays:										
I have to get up at:	08:00	06:00	06:45	06:30	06:30	06:30	08:00	06:00		06:30
I need … minutes to wake up	5	0	15	5	20	5	1	5		1
I regularly wake up … minutes before my alarm	0	15	0	10	0	2	5	0		5
I am fully awake from …	08:05	06:00	08:00	07:15	08:30	06:45	08:30	06:30		07:00
I have an energy dip at around …	16:00	14:00	14:00	15:30	14:00	11:30	17:00	15:00		14:00
On nights before workdays I go to bed at …	00:00	22:00	23:30	22:00	23:00	23:00	00:00	21:30		22:30
It then takes me … minutes to fall asleep	3	15	15	10	10	10	30	30		15
If I can, I like to take a nap (Y/N)	N	N	N	N	Y	N	N	N		N
If Y then I sleep for …	-	-	-	-	7	-	-	-		-
I would feel terrible afterwards (Y/N)	-	-	Y	-	-	-	-	-		-
On free days:										
My dream would be to sleep until …	09:00	08:00	09:00	08:30	09:00	09:30	10:00	07:00		08:30
I normally wake up at …	09:00	06:00	10:30	06:30	07:50	09:00	09:00	06:30		08:00
If I wake up at my workday time I try to get back to sleep (Y/N)	N	N	Y	Y	Y	Y	N	N		Y
If I get back to sleep I will usually sleep for another … minutes	-	-	210	60	60	150	-	20		60
I need … minutes to wake up	5	-	30	5	20	5	-	5		10
I am fully awake from …	09:05	-	11:00	07:15	08:30	09:15	-	07:00		08:30
I have an energy dip at around …	16:00	-	18:00	15:30	14:00	18:00	-	-		-
On nights before free days I go to bed at …	00:30	23:00	01:30	22:30	23:00	00:00	01:00	22:00		23:00
It then takes me … minutes to fall asleep	3	15	30	10	10	15	10	10		30
If I can, I like to take a nap (Y/N)	N	N	N	N	Y	N	N	N		N
If Y then I sleep for …	-	-	-	-	10	-	-	-		-
If Y then I would feel terrible afterwards (Y/N)	-	-	Y	-	-	-	-	-		-
Preferences:										
Once I am in bed, I would like to read for … minutes	0	0	30	30	0	30	20	30		15
… but generally fall asleep after no more than … minutes	-	-	10	20	-	5	20	30		20
I prefer to sleep in a completely dark room (Y/N)	N	Y	Y	Y	Y	Y	Y	Y		Y
I wake up more easily when morning light shines in to my room (Y/N)	Y	Y	Y	Y	Y	Y	Y	Y		Y
How long per day do you spend on average outside exposed to daylight?										
On workdays (hh:mm)	01:30	02:00	1.5	01:00	02:00	03:00	01:30	01:10		01:00
On free days (hh:mm)	02:00	05:30	1.5	03:00	03:30	07:00	03:00	02:00		04:00
Self Assessment										
I am …	3	1	4	1	4	5	5	1		1
As a child I was …	1	2	1	1	4	2	3	0		2
As a teenager I was …	3	4	3	5	5	6	5	6		2
If over 65, in the middle of my life I was …	-	-	-	-	-	-	-	-		-
My mother is/was …	1	1	3	2	3	4	1	4		1
My father is/was …	1	4	6	0	2	5	2	0		0
My sister (1) is/was …	UKW	4	5	-	4	5	6	-		-
My sister (2) is/was …	-	-	6	-	2	4	3	-		-
My sister (3) is/was …	-	-	6	-	-	-	2	-		-
My brother (1) is/was …	-	-	6	4	-	-	5	4		4
My brother (2) is/was …	-	-	6	-	-	-	1	-		3
My brother (3) is/was …	-	-	6	-	-	-	-	-		-
My brother (4) is/was …	-	-	3	-	-	-	-	-		-
My partner (girlfriend/boyfriend, spouse, significant other) is/was …	2	4	0	5	4	5	5	1		-

Computed Variables	Participant									
	1	2	3	4	5	6	7	8	9	10
Sleep onset (WD):	00:03	22:15	23:45	22:10	23:10	23:10	00:30	22:00		22:45
Out of bed (WD):	08:05	06:00	07:00	06:35	06:50	06:35	08:01	06:05		06:31
Sleep duration (WD):	07:57	07:45	07:00	08:20	07:20	07:20	07:30	08:00		07:45
Time in bed (WD):	08:05	08:00	07:30	08:35	07:50	07:35	08:01	08:35		08:01
Mid-sleep (WD):	03:59	02:08	03:15	02:20	02:50	02:50	04:15	02:00		02:38
Sleep onset (FD):	00:33	23:15	02:00	22:40	23:10	00:15	01:10	22:10		23:30
Out of bed (FD):	09:05	06:00	11:00	06:35	08:10	09:05	09:00	06:35		08:10
Sleep duration (FD):	08:27	06:45	08:30	07:50	08:40	08:45	07:50	08:20		08:30
Time in bed (FD):	08:35	07:00	09:30	08:05	09:10	09:05	08:00	08:35		09:10
Mid-sleep (FD):	4:51:00 AM	2:38:00 AM	6:15:00 AM	2:35:00 AM	3:30:00 AM	4:38:00 AM	5:05:00 AM	2:20:00 AM		3:45:00 AM
WD Duration	07:57	07:45	07:00	08:20	07:20	07:20	07:30	08:00		07:45
WD hours	7	7	7	8	7	7	7	8		7
WD mins	57	45	0	20	20	20	30	0		45
WD Weekly Sleep	39.75	38.75	35	41.666667	36.666667	36.666667	37.5	40		38.75
FD Duration	08:27	06:45	08:30	07:50	08:40	08:45	07:50	08:20		08:30
FD hours	8	6	8	7	8	8	7	8		8
FD mins	27	45	30	50	40	45	50	20		30
FD Weekly Sleep	16.9	13.5	17	15.666667	17.333333	17.5	15.666667	16.666667		17
Total Weekly Sleep Time	56.65	52.25	52	57.333333	54	54.166667	53.166667	56.666667		55.75
Total Weekly Sleep Hours	56	52	52	57	54	54	53	56		55
Remainder	0.65	0.25	0	0.33333	0	0.16667	0.16667	0.66667		0.75
Total Sleep Mins	39	15	0	20	0	10	10	40		45
Total Weekly Sleep Time (hh:mm:ss)	56:39:00	52:15:00	52:00:00	57:20:00	54:00:00	54:10:00	53:10:00	56:40:00		55:45:00
Average Daily Sleep duration (hh:mm:ss):	8:05:34	7:27:51	7:25:43	8:11:26	7:42:51	7:44:17	7:35:43	8:05:43		7:57:51
Equation:	1	2	1	2	1	1	1	1		1
Chronotype:	**04:40**	**02:38**	**05:42**	**02:35**	**03:01**	**04:07**	**04:57**	**02:12**		**03:28**

Table S5. Horne-Ostberg Questionnaire

Horne-Ostberg Questionnaire

No.	Statement	Participant Number									
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	Considering only your own "feeling best" rhythm, at what time would you get up if you were entirely free to plan your day?	3	3	3	4	3	3	3	4		3
2	Considering only your own "feeling best" rhythm, at what time would you go to bed if you were entirely free to plan your evening?	3	4	3	3	3	3	2	4		3
3	If there is a specific time you have to get up in the morning, to what extent are you dependent on being woken up by an alarm clock?	2	3	1	3	2	3	2	3		2
4	Assuming adequate environmental conditions, how easy do you find getting up in the morning?	3	4	3	4	3	3	3	3		4
5	How alert do you feel during the first half hour after having woken in the morning?	3	3	3	3	1	2	2	3		3
6	How is your appetite during the first half hour after having woken in the morning?	2	3	2	4	3	2	3	2		3
7	During the first half hour after having woken in the morning, how tired do you feel?	3	3	2	4	2	2	3	3		3
8	When you have no commitments the next day, at what time do you go to bed compared to your usual bedtime?	3	2	2	3	3	2	2	3		3
9	You have decided to engage in some physical exercise. A friend suggests that you do this one hour twice a week and the best time for him is between 0700 and 0800h. Bearing in mind nothing else but your own inclinations, how do you think you would perform?	4	4	3	3	2	2	2	4		3
10	At what time in the evening do you feel tired and as a result in need of sleep?	3	4	3	4	3	3	3	4		3
11	You wish to be at your peak for a test which you know is going to be mentally exhausting and lasting for two hours. You are entirely free to plan your day and considering only your own "feeling best" rhythm, which ONE of the four testing times would you choose?	4	6	6	4	4	2	4	6		4
12	If you went to bed at 2300h at what level of tiredness would you be?	3	2	2	5	3	2	2	5		2
13	For some reason you have gone to bed several hours later than usual, but there is no need to get up at any particular time the next morning. Which ONE of the following events are you most likely to experience:	1	3	3	4	3	2	2	4		3
14	One night you have to remain awake between 0400 and 0600h. You have no commitments the next day. Which ONE of the following suits you best:	3	4	4	3	3	1	3	4		4
15	You have to do two hours of hard physical work. You are entirely free to plan your day and considering only your own "feeling best" rhythm which ONE of the following times would you choose?	3	4	3	3	3	1	2	4		3
16	You have decided to engage in some physical exercise. A friend suggests that you do this between 2200 and 2300h twice a week. Bearing in mind nothing else but your own "feeling best" rhythm how well do you think you would perform?	1	4	2	4	2	1	2	3		3
17	Suppose that you can choose your own work hours. Assume that you worked a FIVE hour day (including breaks)) and that your job was interesting and paid by results. Which FIVE CONSECUTIVE HOURS would you select:	2	3	2	3	2	2	2	3		3
18	At what time of day do you think that you reach your "feeling best" peak?	3	4	5	3	3	2	3	4		3
19	One hears of "morning" and "evening" types. Which do you consider yourself to be?	4	4	2	6	4	0	2	6		6
	Total Score	53	67	54	70	52	38	47	72		61
	Category	Neither	M. Morn	Neither	D. Morn	Neither	M. Even	Neither	D. Morn		M. Morn

Table S6. Pittsburgh Sleep Quality Index

Pittsburgh Sleep Quality Index

			Participant									
Question			**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
	1	a	12:20:00 AM	10:00:00 PM	11:00:00 PM	10:00:00 PM	11:10:00 PM	11:00:00 PM	12:00:00 AM	10:00:00 PM		10:30:00 PM
	2	a	3	15	12.5	10	10	5	30	5		15
LATEN		score	0	0	0	0	0	0	1	0		0
	3	a	9:00:00 AM	6:30:00 AM	7:00:00 AM	6:30:00 AM	6:35:00 AM	9:15:00 AM	8:00:00 AM	6:00:00 AM		6:20:00 AM
		diff	08:40	08:30	08:00	08:30	07:25	10:15	08:00	08:00		07:50
		hr	8	8	8	8	7	10	8	8		7
		min	40	30	0	30	25	15	0	0		50
		sec	31200	30600	28800	30600	26700	36900	28800	28800		28200
		diffhour	8.67	8.50	8.00	8.50	7.42	10.25	8.00	8.00		7.83
		newtib	8.67	8.50	8.00	8.50	7.42	10.25	8.00	8.00		7.83
		tmphse	99	82	81	82	94	98	94	100		89
4	**HSE**	**score**	**0**	**1**	**1**	**1**	**0**	**0**	**0**	**0**		**0**
	4	a	08:37	07:00	06:30	07:00	07:00	10:00	07:30	08:00		07:00
		hr	8	7	6	7	7	10	7	8		7
		min	37	0	30	0	0	0	30	0		0
		sec	31020	25200	23400	25200	25200	36000	27000	28800		25200
			8.62	7.00	6.50	7.00	7.00	10.00	7.50	8.00		7.00
3	**DURAT**	**score**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**		**0**
	5	a	0	1	0	1	1	0	2	1		0
		2a + 5a	0	1	0	1	1	0	3	1		0
2	**LATEN**	**score**	**0**	**1**	**0**	**1**	**1**	**0**	**2**	**1**		**0**
		b	3	2	0	3	3	2	1	0		2
		c	0	2	0	1	3	1	2	0		2
		d	0	0	0	0	0	0	1	0		0
		e	0	0	0	0	0	0	1	0		0
		f	0	0	0	0	0	1	1	0		0
		g	0	2	0	2	1	1	0	1		2
		h	0	0	2	1	0	0	0	0		0
		i	0	1	0	0	0	0	0	0		0
		j	0	0	0	0	0	2	0	0		0
		sum b-j	3	7	2	7	7	7	6	1		6
5	**DISTB**	**score**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**		**1**
1	**6**	**a**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**0**		**1**
6	**7**	**a**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**		**0**
	8	a	0	1	0	0	0	0	0	0		0
	9	a	0	1	1	0	0	0	2	0		0
		8 + 9	0	2	1	0	0	0	2	0		0
7	**DAYDYS**	**score**	**0**	**1**	**1**	**0**	**0**	**0**	**1**	**0**		**0**
	10		3	3	0	0	0	3	0	3		1
		a	0	0	0	0	0	0	0	0		0
		b	0	0	0	0	0	0	0	0		0
		c	0	0	0	0	0	0	0	0		0
		d	0	0	0	0	0	0	0	0		0
		e	0	0	0	0	1	2	0	0		0
PSQI		**Score**	**1**	**5**	**5**	**4**	**3**	**2**	**5**	**2**	-	**2**
		Interp	**Good**	**Good**	**Good**	**Good**	**Good**	**Good**	**Good**	**Good**	-	**Good**

Supplementary Figures

Figure S1

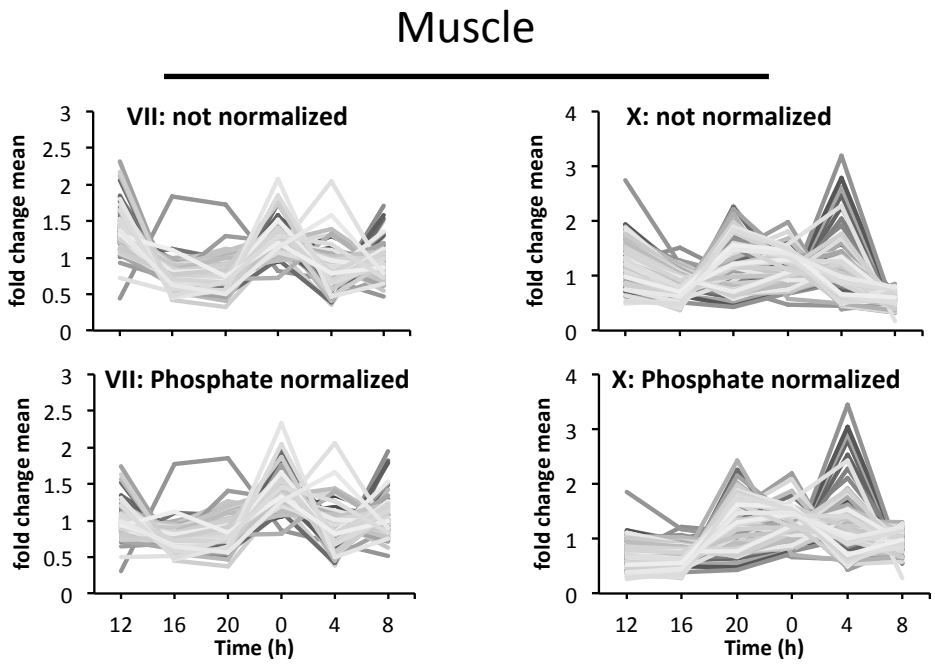


Figure S2

Logarithmic scale heatmap of lipid concentration for *in vitro* (left) and *in vivo* (right)

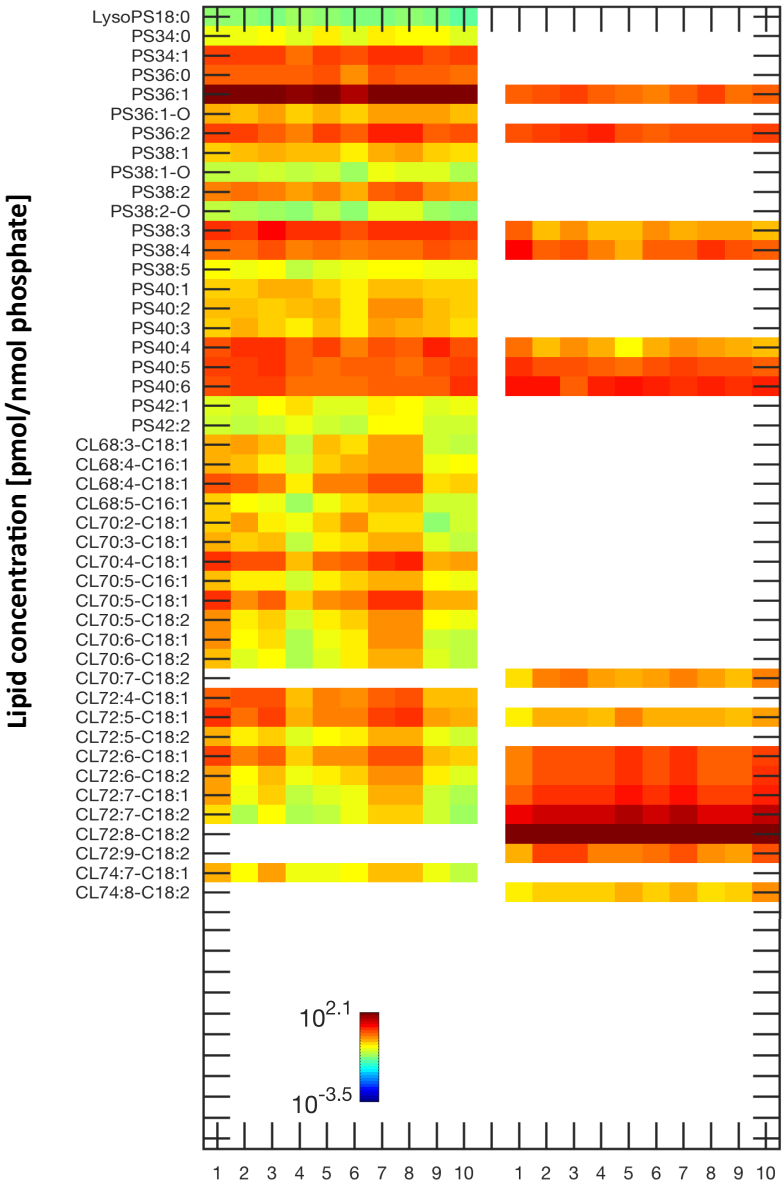


Figure S3

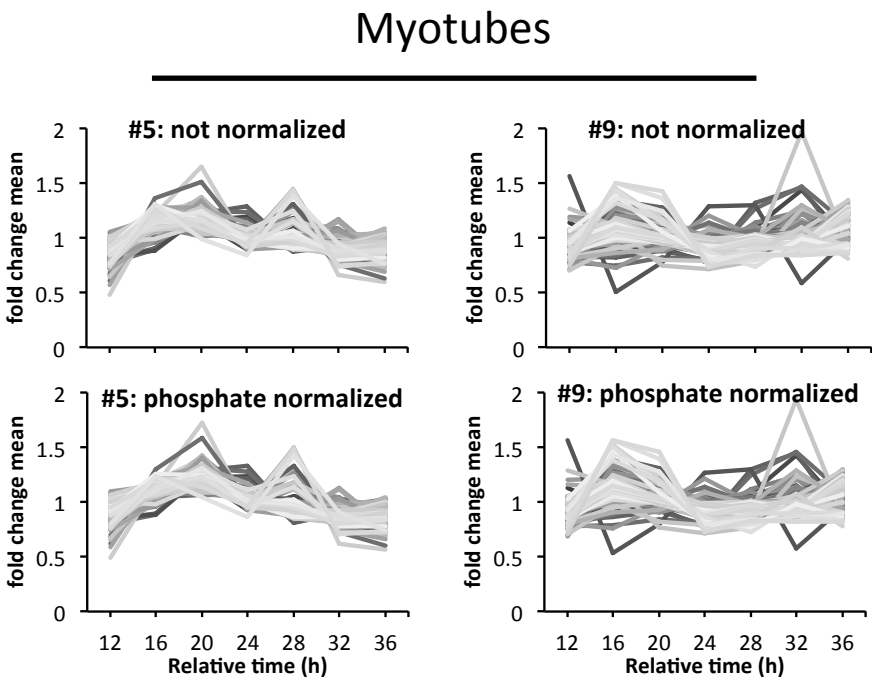

Myotubes
#5: not normalized
#9: not normalized
#5: phosphate normalized
#9: phosphate normalized
fold change mean
Relative time (h)
0
0.5
1
1.5
2
12
16
20
24
28
32
36

Figure S4

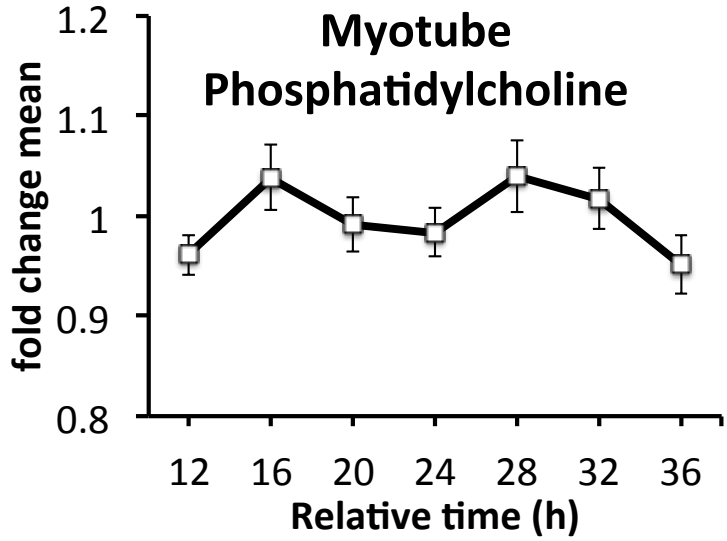

Myotube
Phosphatidylcholine
fold change mean
1.2
1.1
1
0.9
0.8
12
16
20
24
28
32
36
Relative time (h)

Figure S5

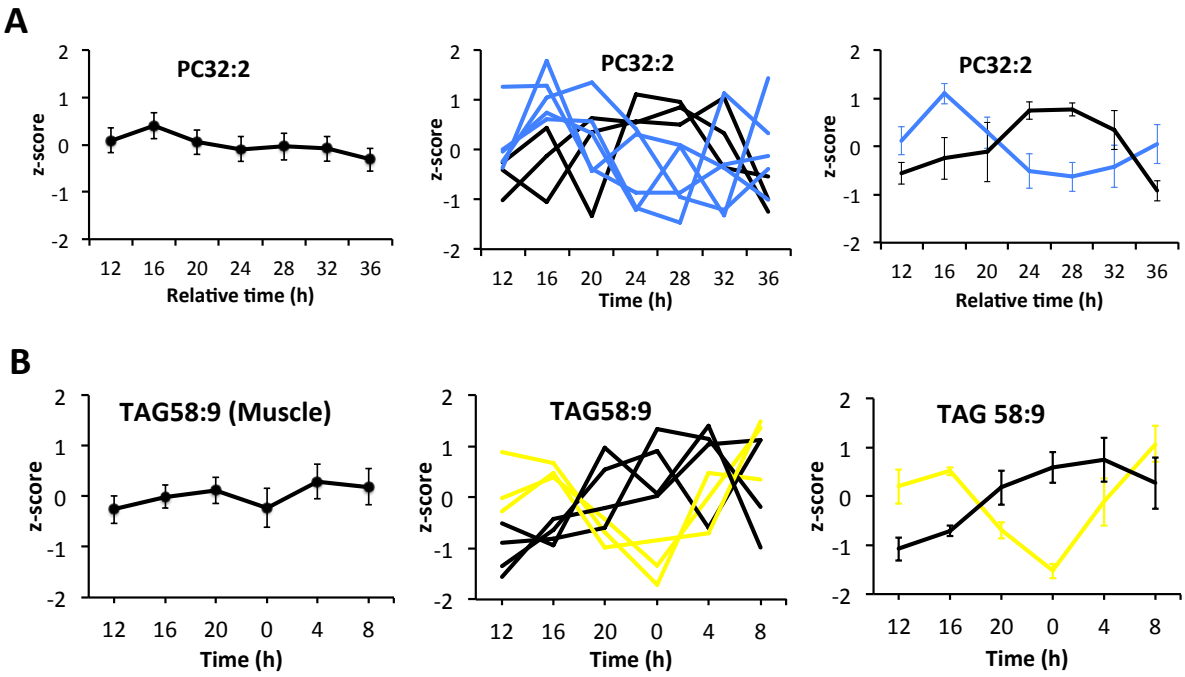

A
PC32:2
z-score
Relative time (h)
PC32:2
Time (h)
PC32:2
Relative time (h)
B
TAG58:9 (Muscle)
Time (h)
TAG58:9
Time (h)
TAG 58:9
Time (h)

Figure S6

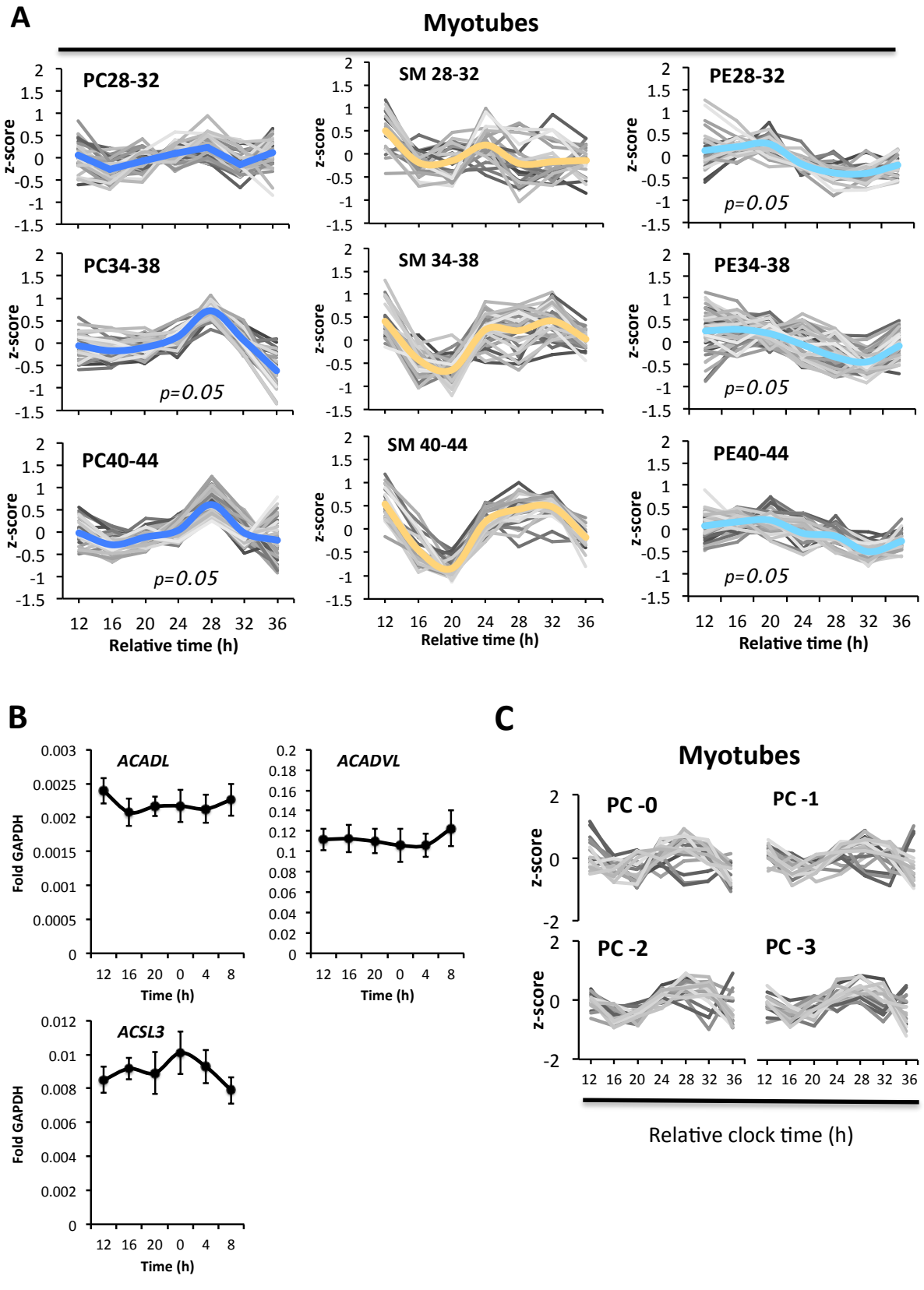
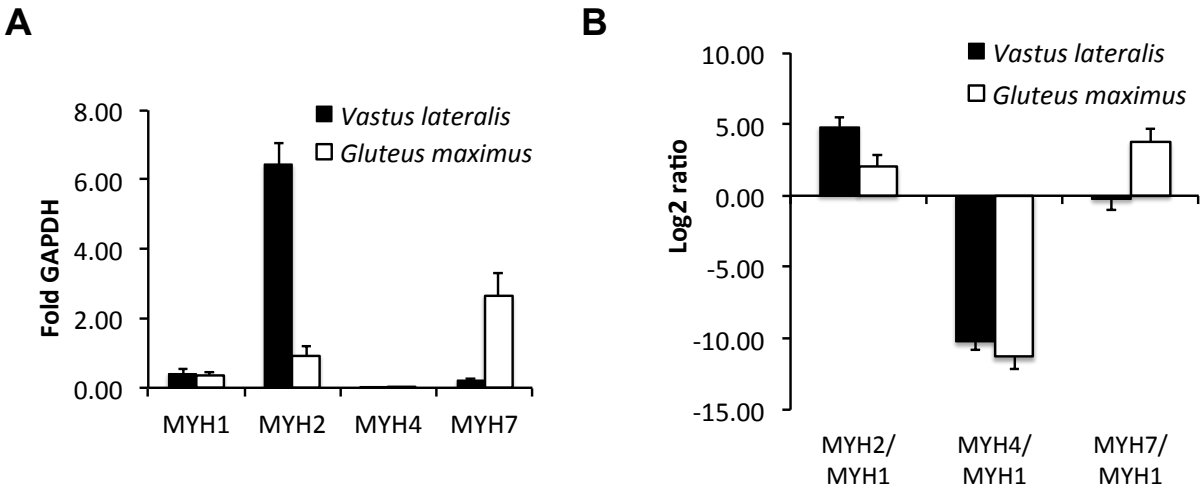

A
Myotubes
PC28-32
SM 28-32
PE28-32
PC34-38
SM 34-38
PE34-38
PC40-44
SM 40-44
PE40-44
p=0.05
z-score
Relative time (h)
B
ACADL
ACADVL
ACSL3
Fold GAPDH
Time (h)
C
Myotubes
PC -0
PC -1
PC -2
PC -3
z-score
Relative clock time (h)

Figure S7


A
8.00
6.00
4.00
2.00
0.00
Fold GAPDH
Vastus lateralis
Gluteus maximus
MYH1
MYH2
MYH4
MYH7
B
10.00
5.00
0.00
-5.00
-10.00
-15.00
Log2 ratio
Vastus lateralis
Gluteus maximus
MYH2/ MYH1
MYH4/ MYH1
MYH7/ MYH1