

Faculty Disclosure Information Elements

Name of Faculty Garret FitzGerald

Title of Presentation Molecular Clocks

Name of Commercial Interest No relevant financial relationship exists

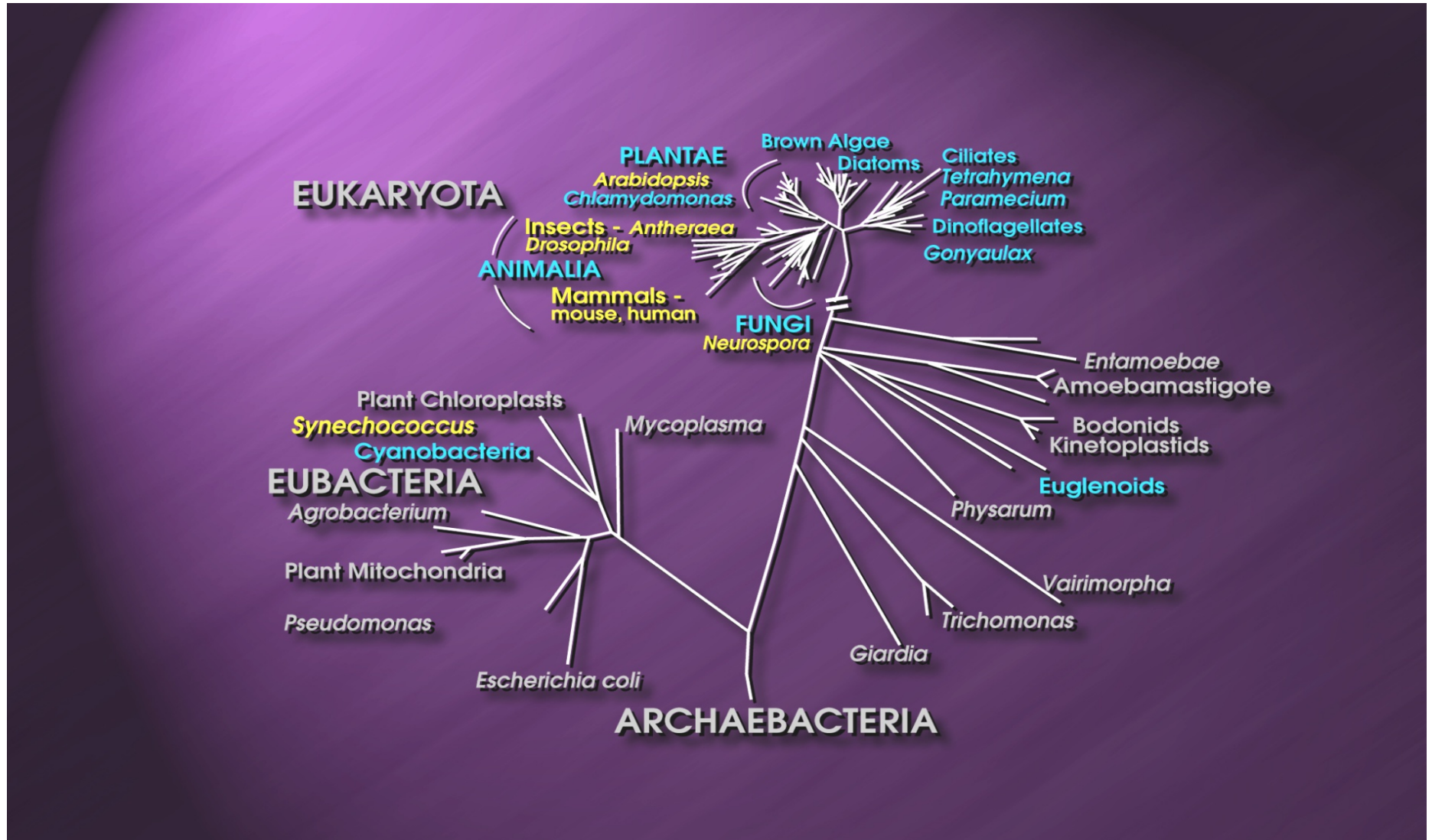
Unwinding the Clock in Cardio- Metabolic Disease

ATVB Meeting
Nashville

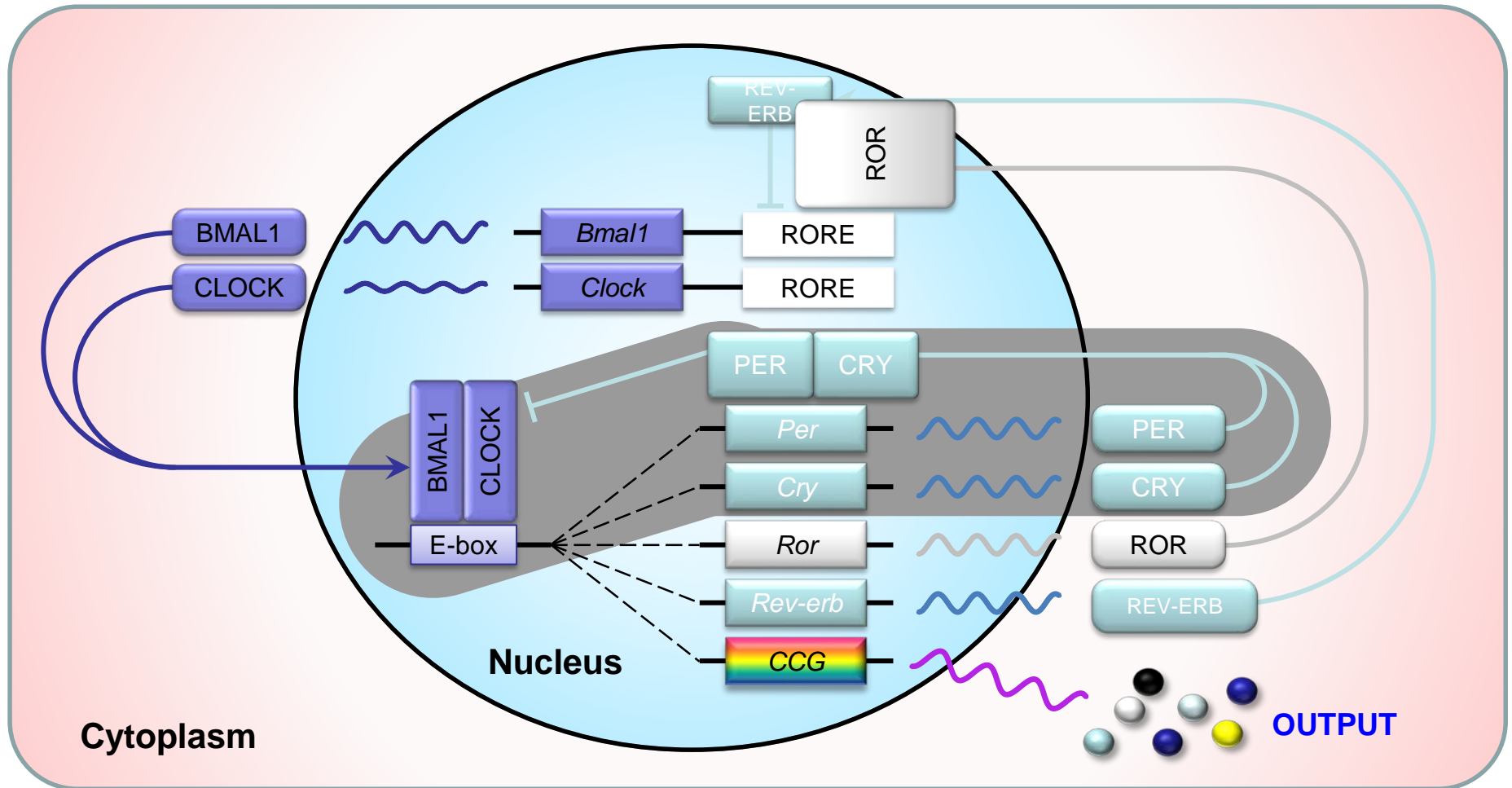
Clock fundamentals

- Master clock in the Supra Chiasmatic Nucleus
- Clocks in all peripheral tissues except the testis
- Diverse signals from the center can entrain the periphery which retains a capacity for autonomy
- Environmental cues adjust the clock to a 24 hour rhythm – light, temperature, food
- It's an anticipatory system
- But why should you care.....?

Clocks are highly conserved in evolution



Clockworks are tightly regulated

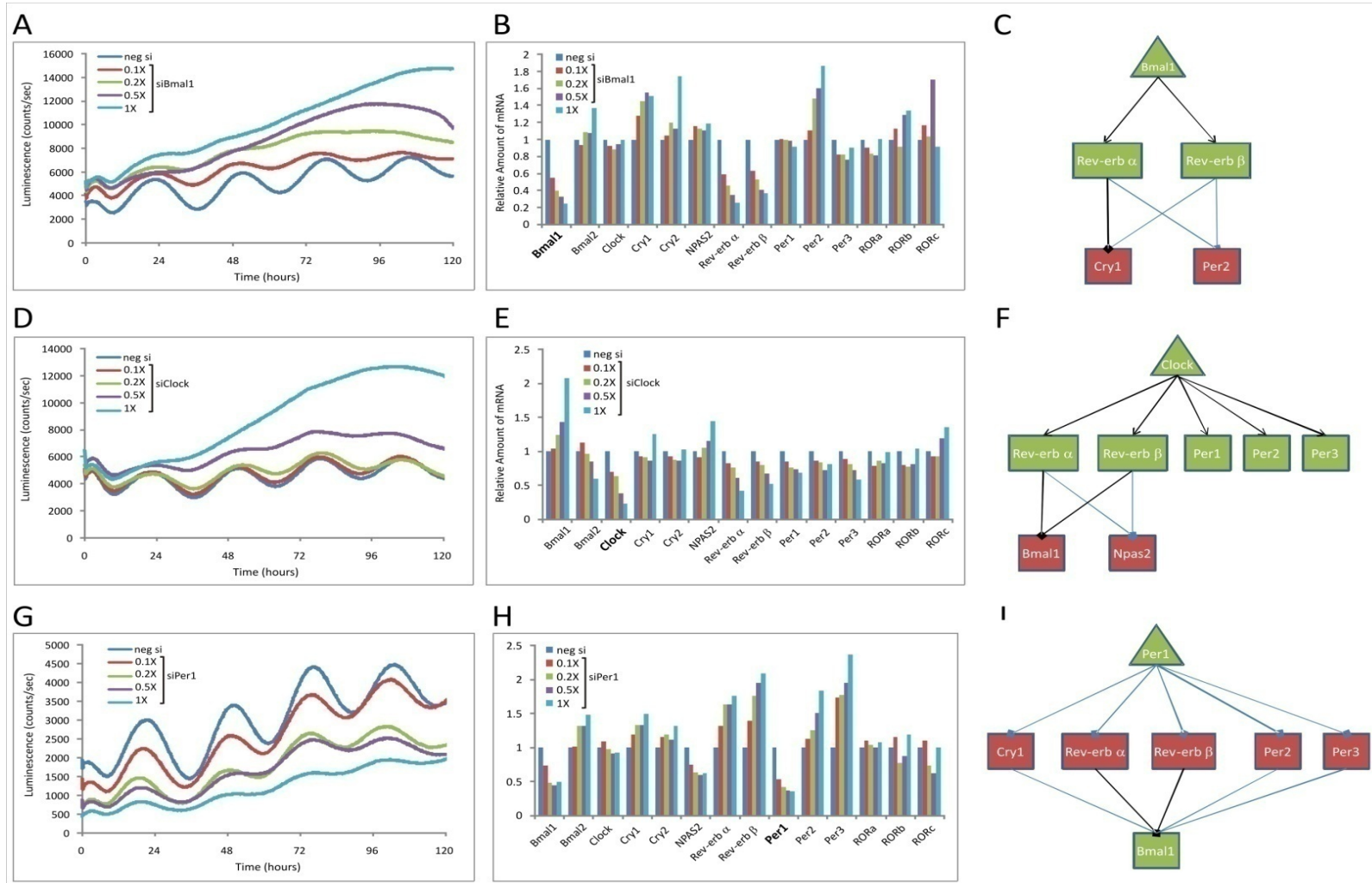


Yang et al Sci Trans Med 5;212rv3, 2013

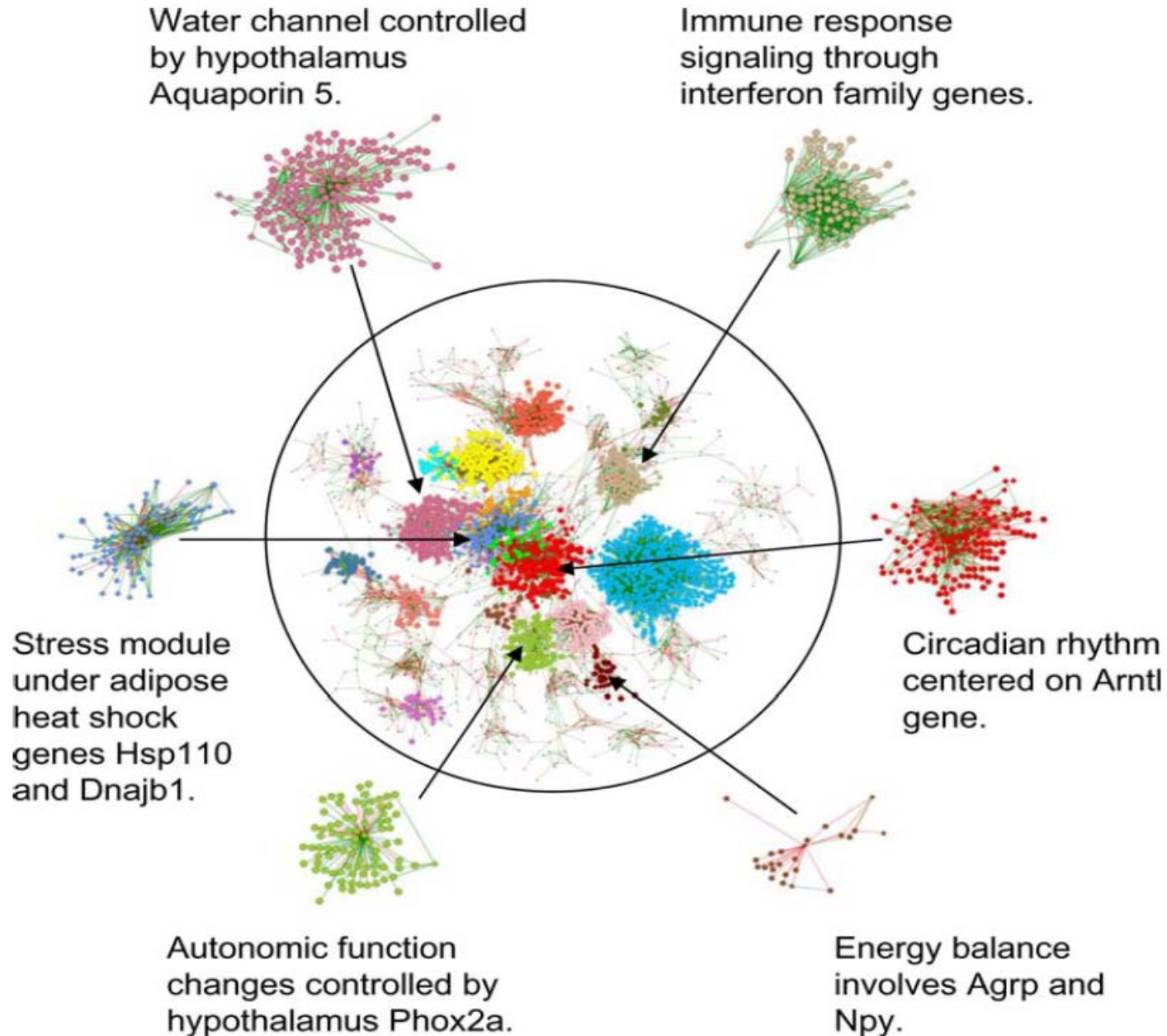
Redundancy is built in to a highly regulated System

siRNA KD defines Network Features of Mammalian Clock

Bmal – Luc Oscillation



Clockworks knit together Biological Networks across Tissues.



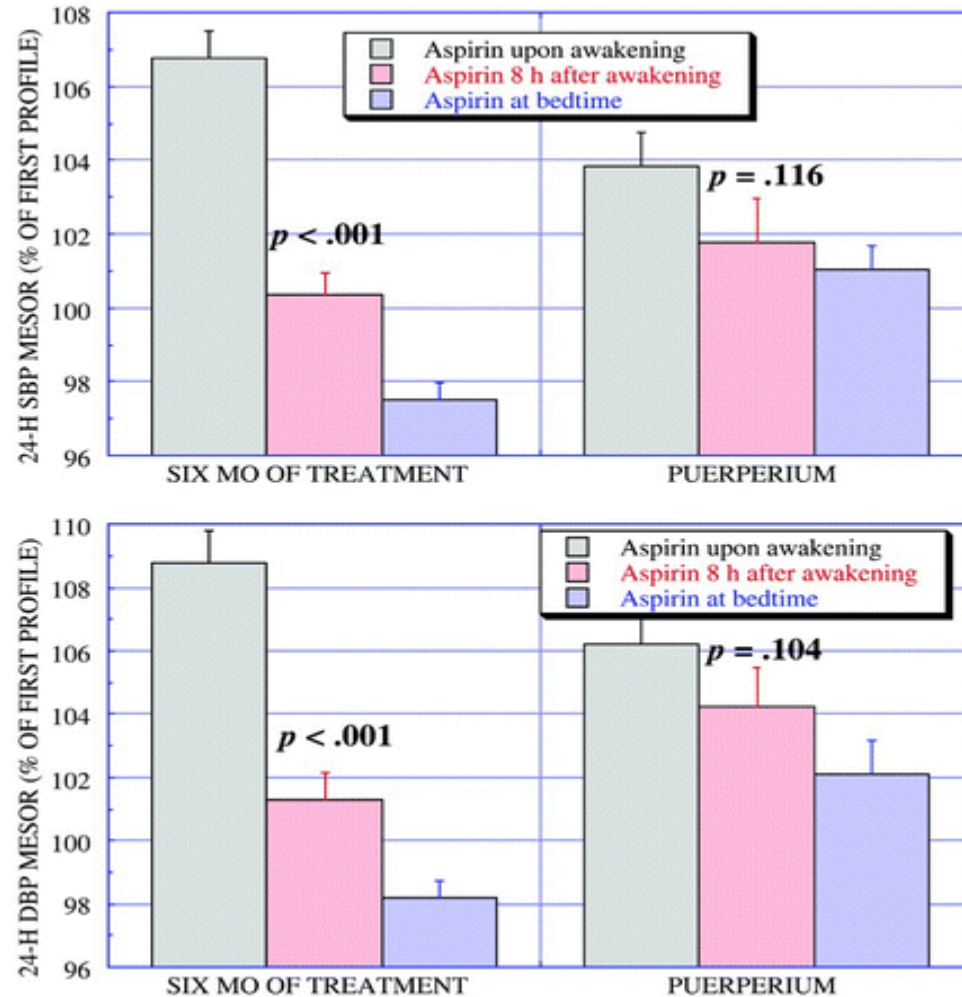
E. Schadt

Clock-regulated drug targets

<u>Rank</u>	<u>Sales</u>	<u>Trade name</u>	<u>Drug</u>	<u>Indications</u>	<u>Circadian-gene targets</u>	<u>Organs in which targets oscillate</u>
2	\$1.46 b	Nexium	esomeprazole	Gastritis, GERD, Esophagitis	<i>Atp4a</i>	liver
5	\$1.28 b	Advair Diskus	fluticasone + salmeterol	Asthma, Chronic obstructive pulmonary di...	<i>Adrb2</i>	kidney, lung, skeletal muscle
11	\$794 m	Rituxan	rituximab	Rheumatoid arthritis, Non-Hodgkin's lymph...	<i>Fcgr2b, Ms4a1</i>	kidney, skeletal muscle
20	\$538 m	Diovan	valsartan + hydrochlorothiazide	Hypertension, Heart failure	<i>Kcnma1</i>	liver
27	\$431 m	Vyvanse	lisdexamfetamine	Attention deficit hyperactivity disorder	<i>Adra1b</i>	liver
32	\$392 m	Tamiflu	oseltamivir	Influenza	<i>Neu1, Neu2</i>	liver, kidney, lung, cerebellum
33	\$383 m	Ritalin	methylphenidate	Attention deficit hyperactivity disorder	<i>Slc6a4</i>	kidney, adrenal gland
37	\$348 m	AndroGel	testosterone	Hypogonadism	<i>Ar</i>	brown fat, aorta, brainstem
38	\$346 m	Lidoderm	lidocaine	Pain	<i>Egfr</i>	heart
44	\$304 m	Seroquel XR	quetiapine	Bipolar disorder, Major depressive disorder...	<i>Htr2a, Drd4, Htr2c, ...</i>	liver, kidney, lung, brown fat, heart, a...
45	\$289 m	Viagra	sildenafil	Erectile dysfunction	<i>Pde5a, Pde6g</i>	brown fat, adrenal gland
47	\$281 m	Niaspan	niacin	Hyperlipidemia	<i>Qprt</i>	kidney
48	\$279 m	Humalog	insulin lispro	Diabetes mellitus T2	<i>Igf1r</i>	kidney
49	\$274 m	Alimta	pemetrexed	Mesothelioma, Non-small cell lung cancer	<i>Tyms, Gart, Atic</i>	liver, lung, aorta
54	\$267 m	Combivent	ipratropium bromide + salbutamol	Asthma, Chronic obstructive pulmonary di...	<i>Chrm2, Adrb2, Adrb1</i>	kidney, lung, heart, skeletal muscle, br...
56	\$262 m	ProAir HFA	salbutamol	Asthma, Chronic obstructive pulmonary di...	<i>Adrb2, Adrb1</i>	kidney, lung, skeletal muscle
62	\$240 m	Janumet	metformin + sitagliptin	Diabetes mellitus T2	<i>Prkab1, Dpp4</i>	kidney, heart, brainstem, hypothalamus
66	\$236 m	Toprol XL	metoprolol	Hypertension, Heart failure	<i>Adrb2, Adrb1</i>	kidney, lung, skeletal muscle
71	\$220 m	Vytorin	ezetimibe + simvastatin	Hyperlipidemia	<i>Anpep, Soat1, Hmgcr</i>	liver, lung, brainstem
78	\$209 m	Aciphex	rabeprazole	Gastritis, GERD, Esophagitis	<i>Atp4a</i>	liver
90	\$189 m	Lunesta	eszopiclone	Insomnia	<i>Tspo, Gabra3</i>	kidney, lung, adrenal gland
98	\$173 m	Prilosec	omeprazole	Gastritis, GERD, Esophagitis	<i>Atp4a</i>	liver
99	\$171 m	Focalin XR	dexmethylphenidate	Attention deficit hyperactivity disorder	<i>Slc6a4</i>	kidney, adrenal gland

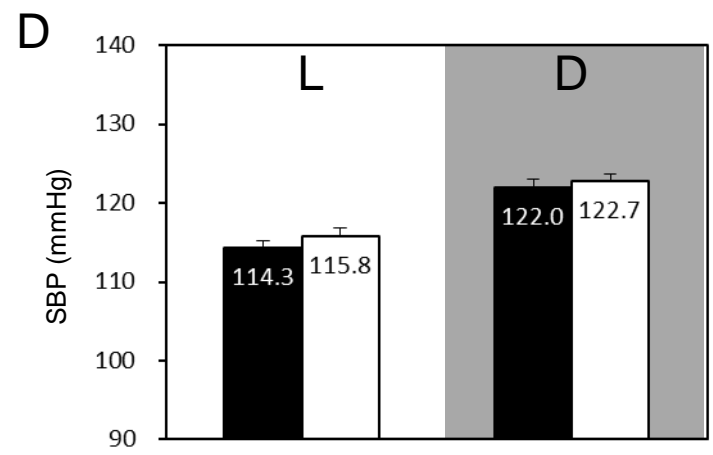
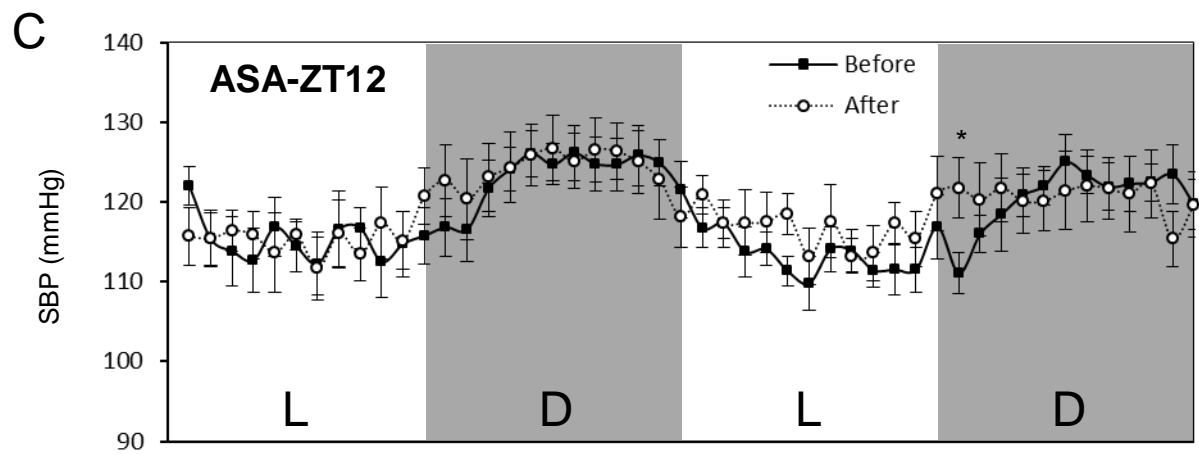
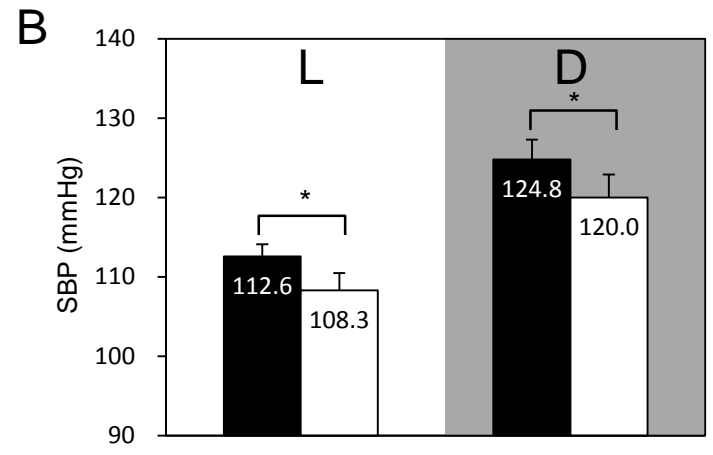
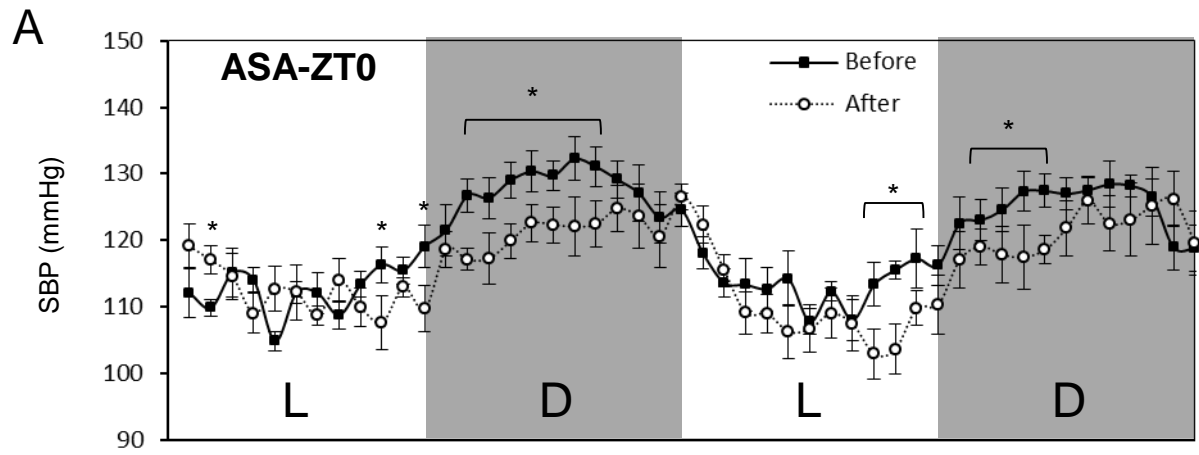
Table 1: Drugs from the top-100 best-selling drugs list that target circadian genes AND have half-life < 6h. For full table, see Dataset S1. Rank and sales are based on USA 2013 Q1 data from Drugs.com.

Time dependent hypotensive effect of low dose aspirin



Time dependent hypotensive effect of low dose aspirin in mice on HSD

Before ASA treatment
 After ASA treatment

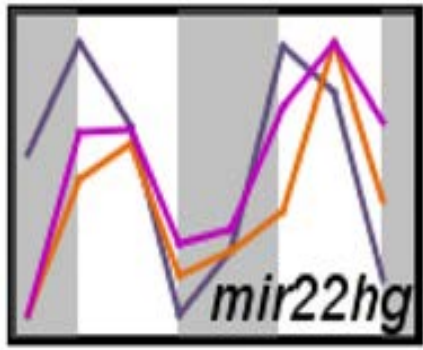
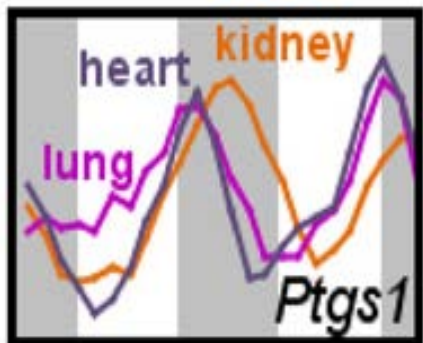


A circadian gene expression atlas in mammals: Implications for biology and medicine

Ray Zhang^{a,1}, Nicholas F. Lahens^{a,1}, Heather I. Ballance^a, Michael E. Hughes^{b,2}, and John B. Hogenesch^{a,2}

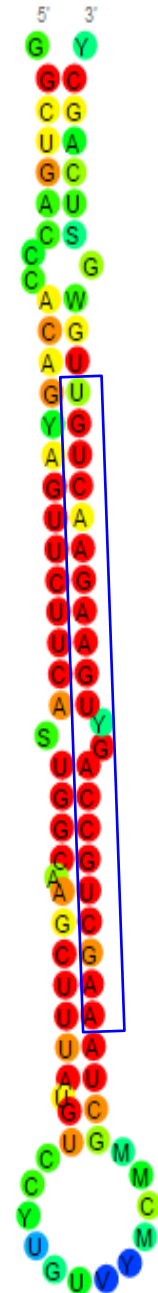
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Edited by Joseph S. Takahashi, Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, TX, and approved September 19, 2014 (received for review May 13, 2014)



Ptgs1 (COX-1)

mir22 →



1.00
0.00
Sequence Conservation

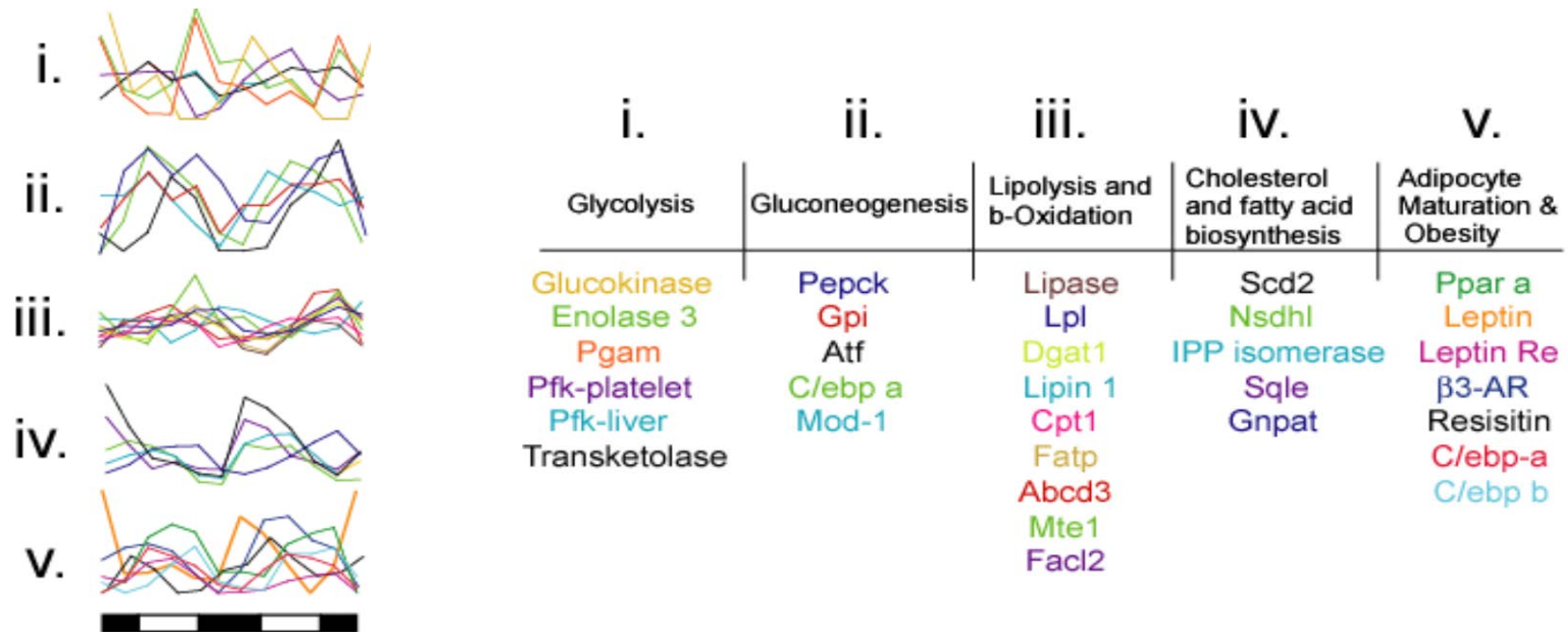
How we were...

- Clear distinction between light and dark
- Clear thermal contrasts
- We ate, quickly, what we killed

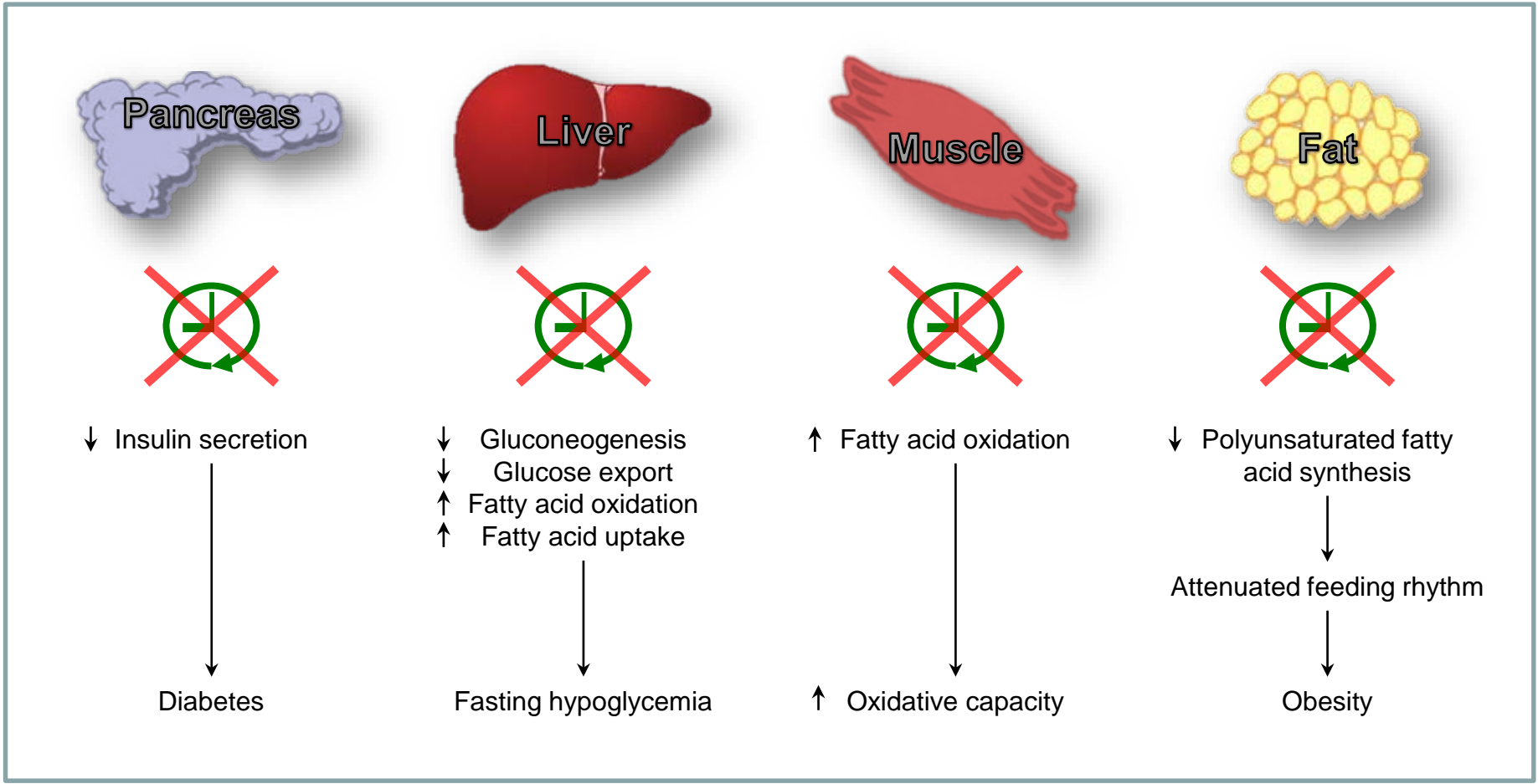
How we are...

- Continuous access to food
- Living in controlled thermal environments
- Jet lag, shift work
- Night lights, cell phones, tablets...

Oscillator subsets in Aortic Tissue

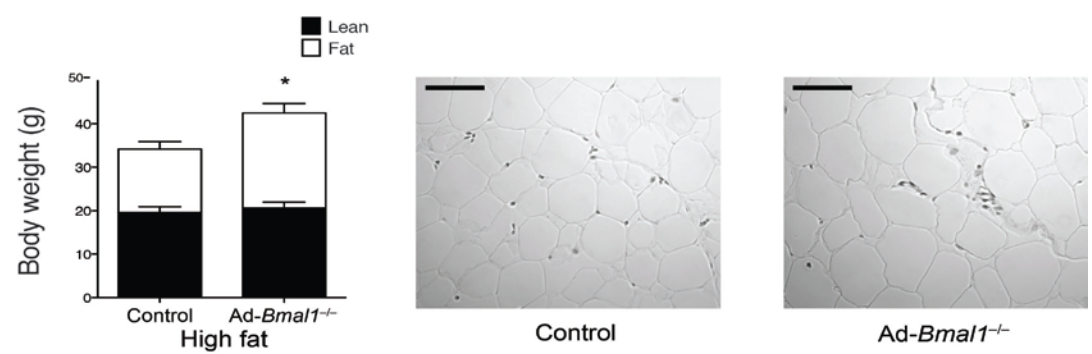
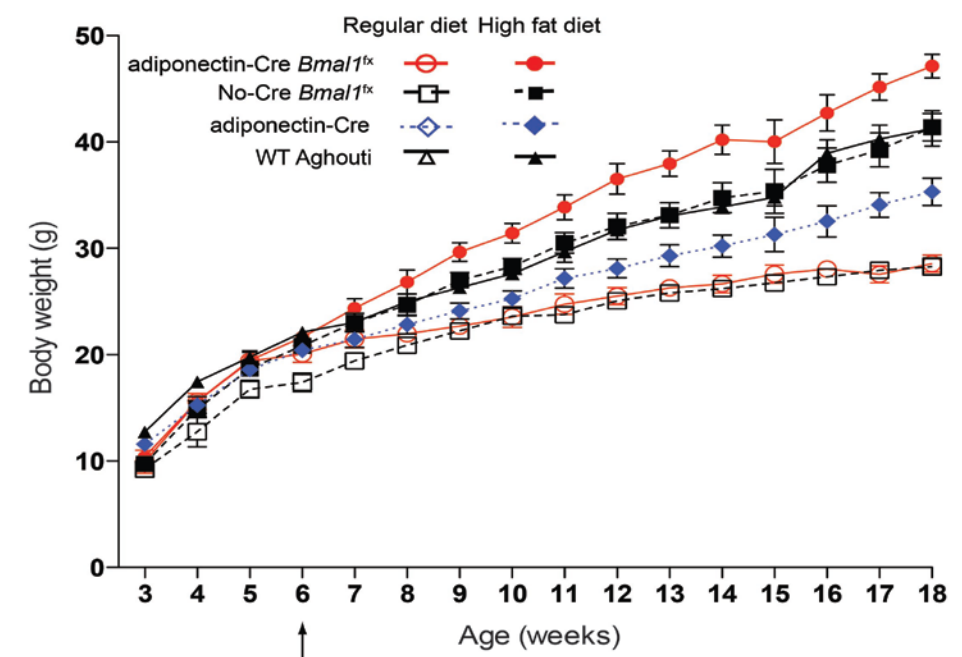
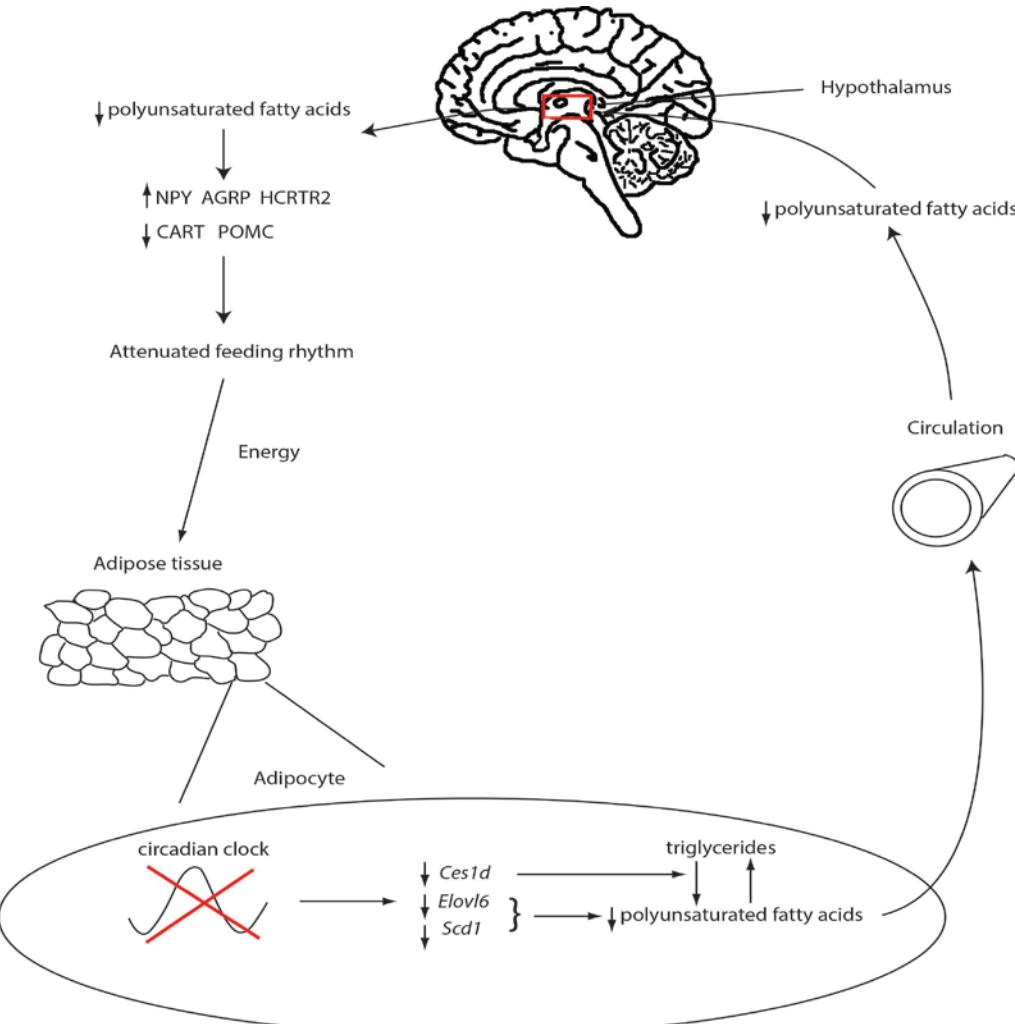


Rudic et al Circulation 112 :2716-24, 2005.

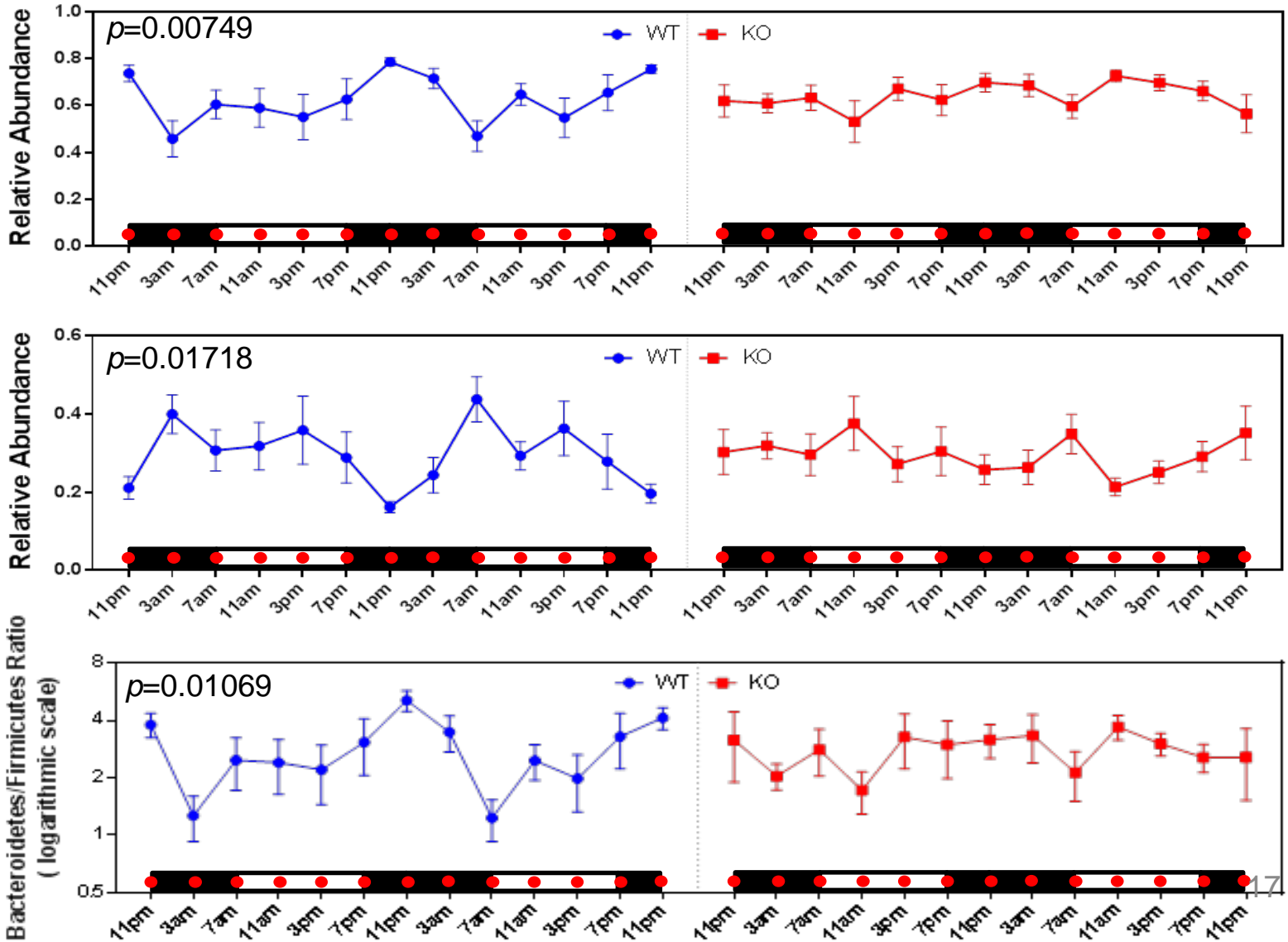


Yang et al Sci Trans Med 5;212rv3, 2013

Peripheral Regulation of Central Behavior



Bmal1 deletion abolishes oscillations in microbial abundances

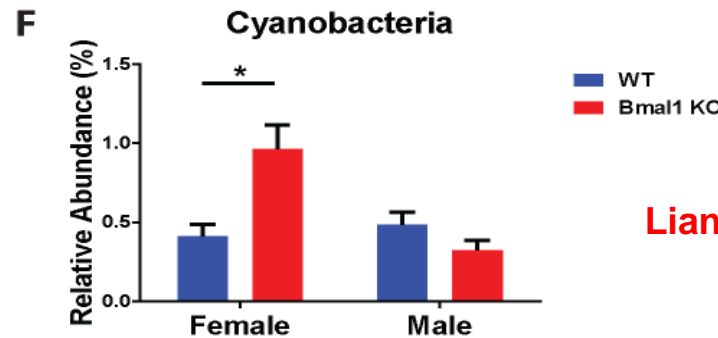
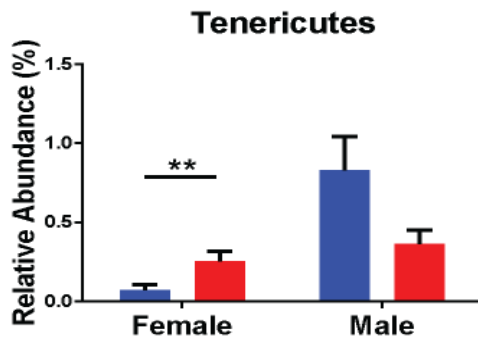
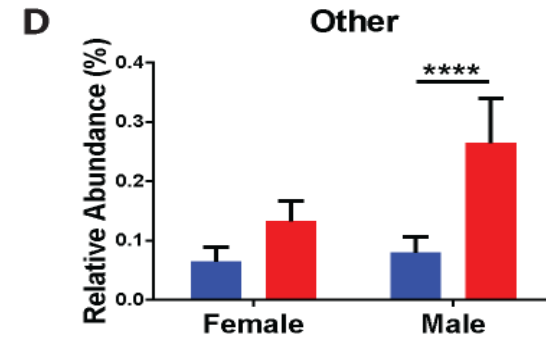
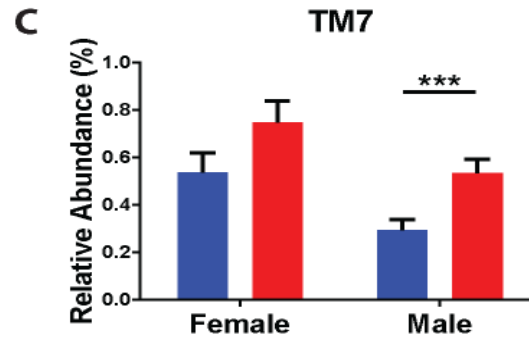
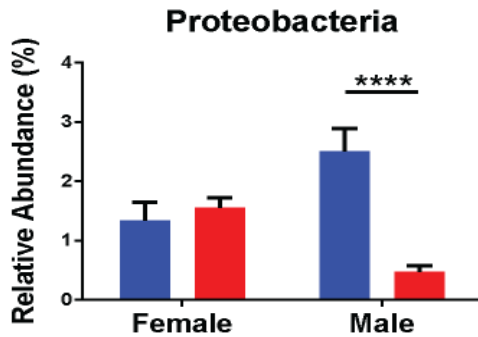
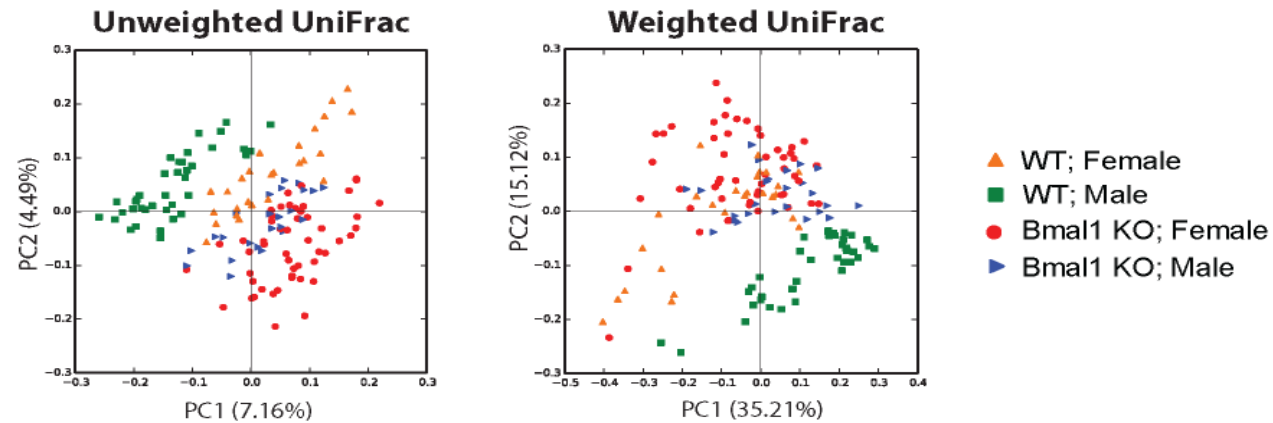


Bacteroidetes

Firmicutes

B/F Ratio

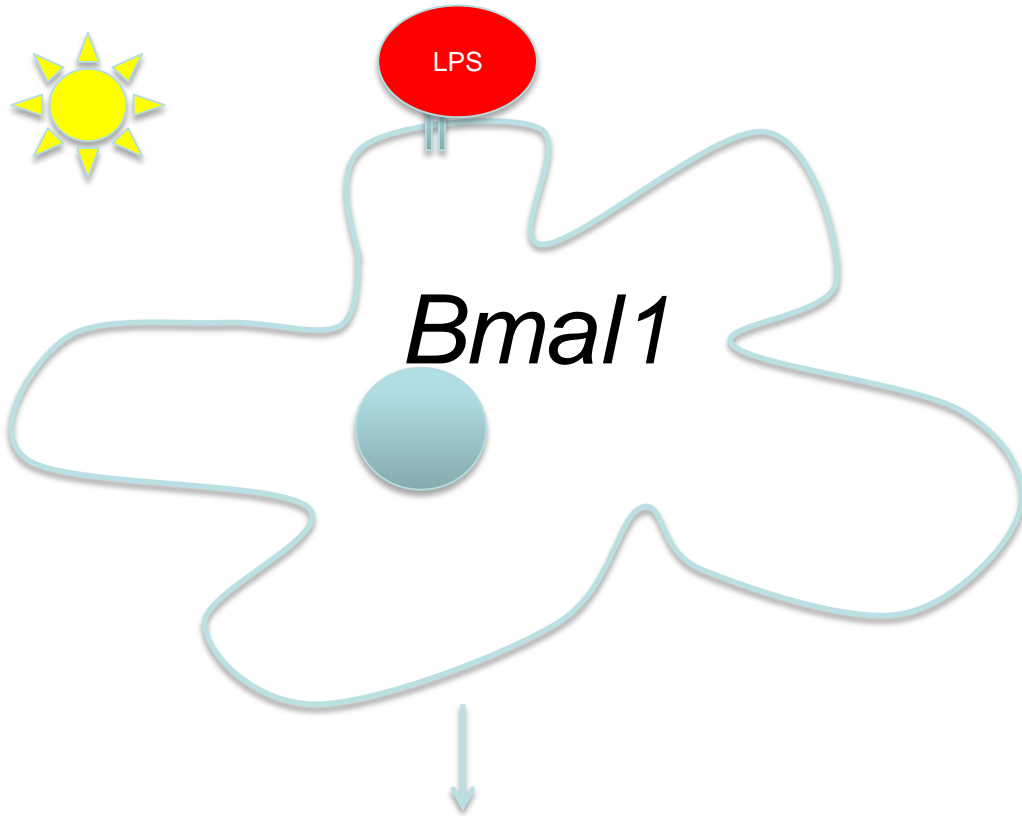
Gender dependent impact of clock disruption on bacterial abundance



Liang et al PNAS 12(33):10479-84, 2015

The temporal levels of Bmal1 in a macrophage counters inflammation in a time dependent manner

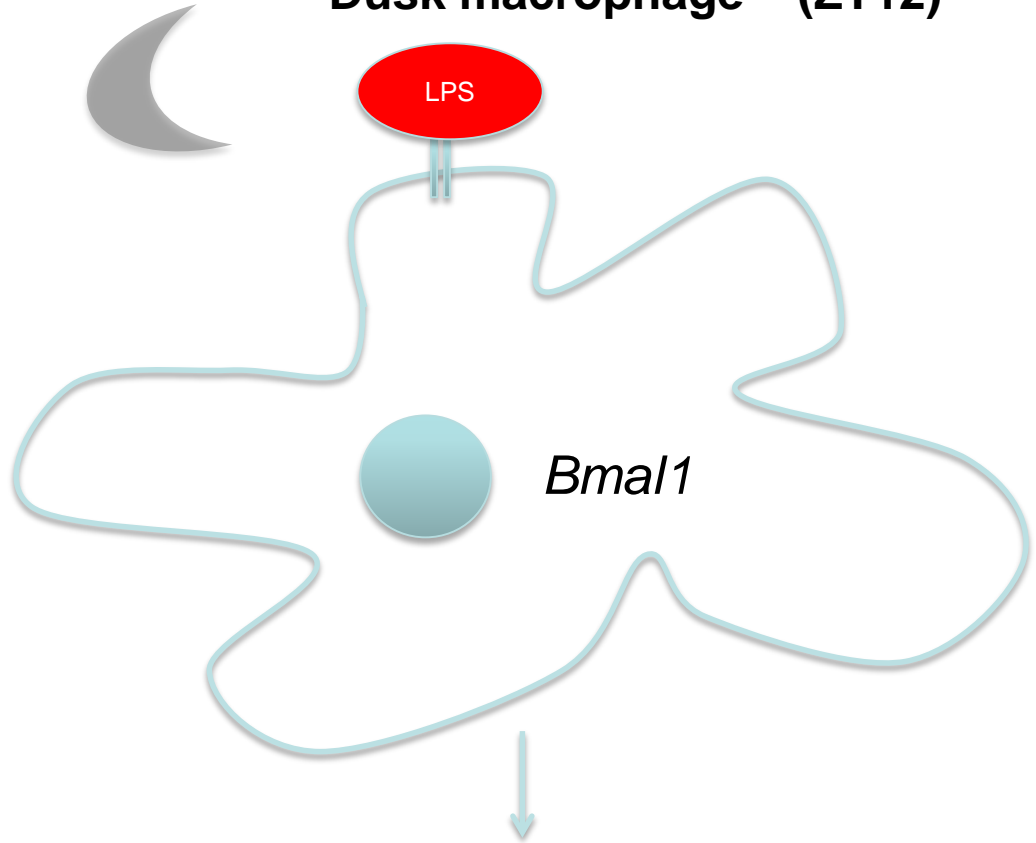
Dawn macrophage (ZT0)



Less cytokines and chemokines

Less sepsis

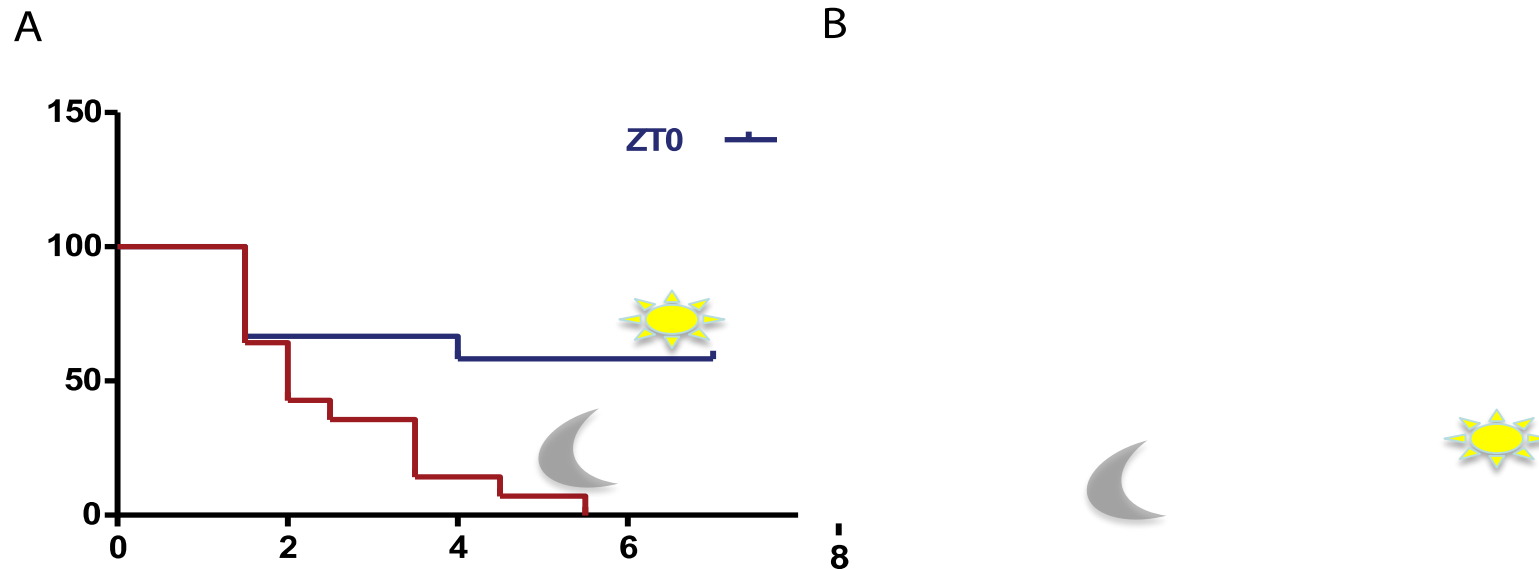
Dusk macrophage (ZT12)



More cytokines and chemokines

More sepsis

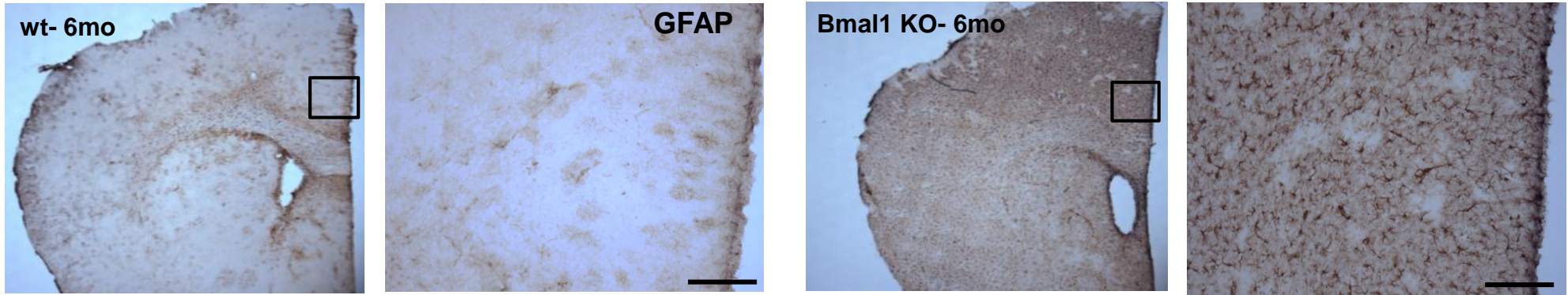
BMAL1 in the myeloid lineage is responsible for the morning protection of LPS



Curtis et al PNAS 2015 Jun 9;112(23):7231-6

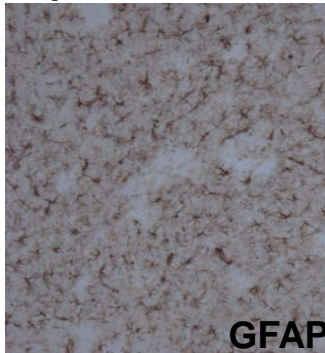
ZT0 = DAWN = *Bmal1* high
ZT12 = DUSK = *Bmal1* low

Disruption of positive limb function causes neuropathology



- BMAL1 KO:**
- Severe astroglisis
 - Late microglial activation
 - Synaptic degeneration
 - Impaired neural network function

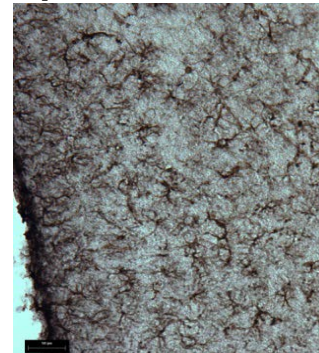
Npas2 KO



Clock KO



Npas2/Clock DKO



Bmal1 KO

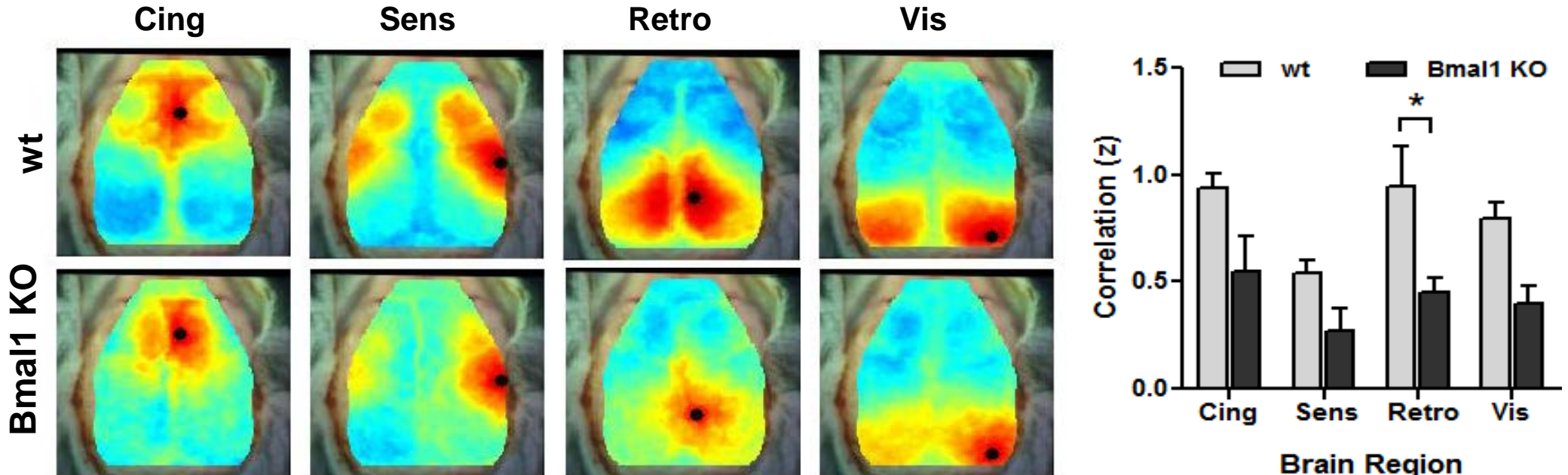


Per1^m/Per2^m



Functional connectivity deficits in *BMAL1* KO mice

Resting-state functional connectivity optical intrinsic signal imaging (fcOIS)-Culver lab



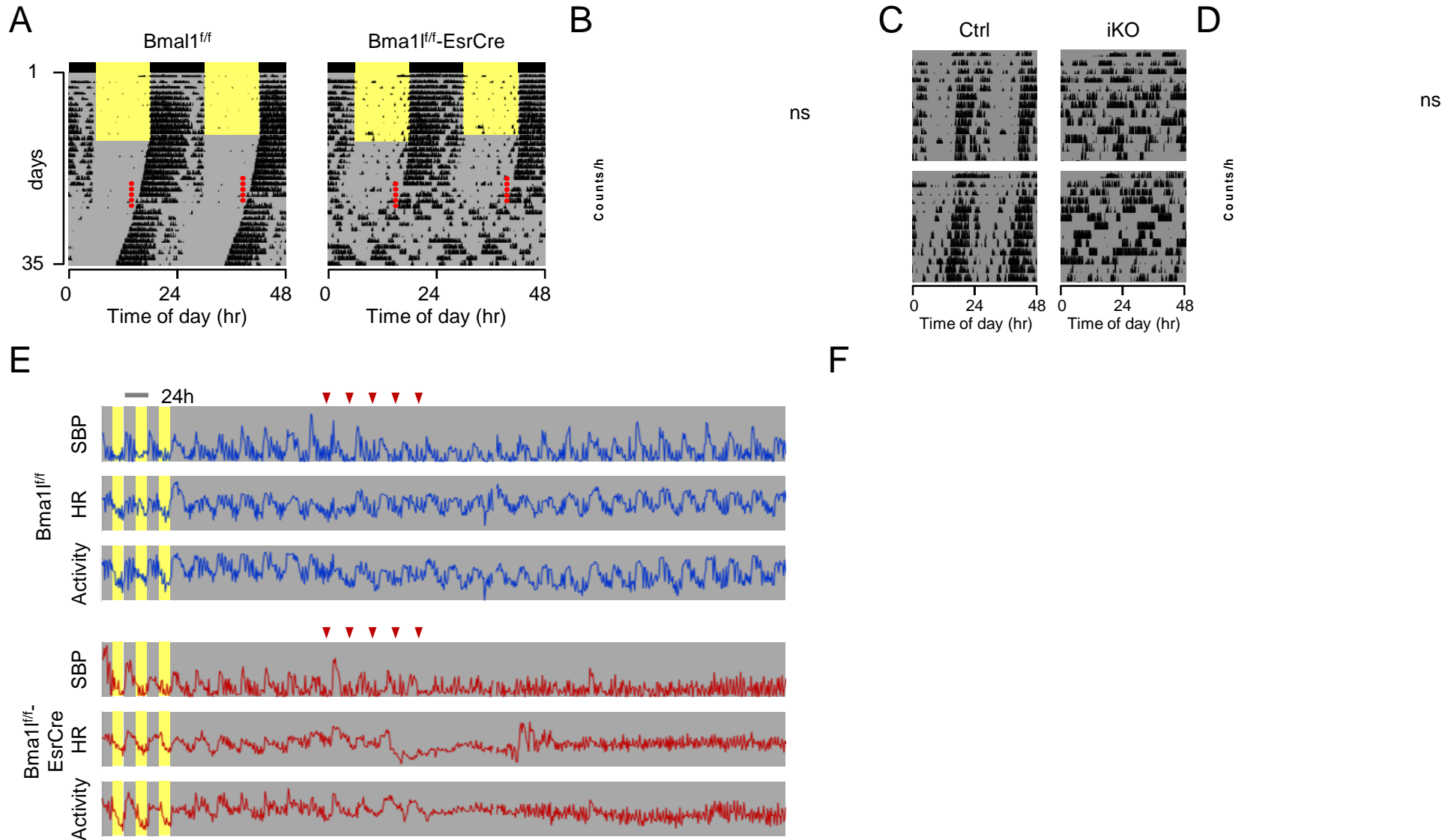
Musiek et al J.Clin. Invest.123(12):5389–54002013

Disordered clocks...

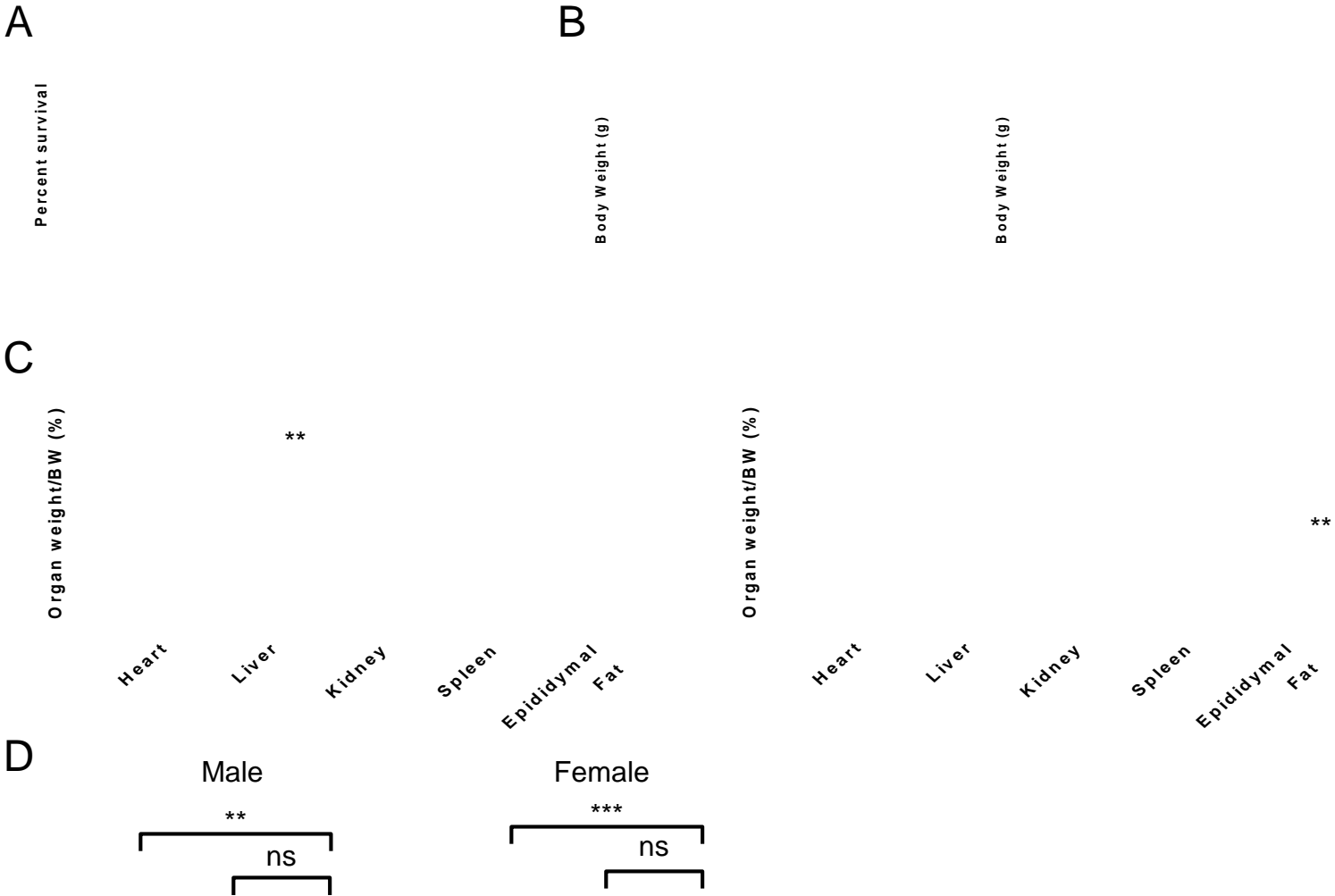
- Metabolic dysfunction
- Disrupted immunoregulation
- Neurodegeneration and accelerated aging

Based largely on deletion of the one non-redundant core clock gene, *Bmal1*. Conventional knockouts have a lifespan roughly 1/3 of normal

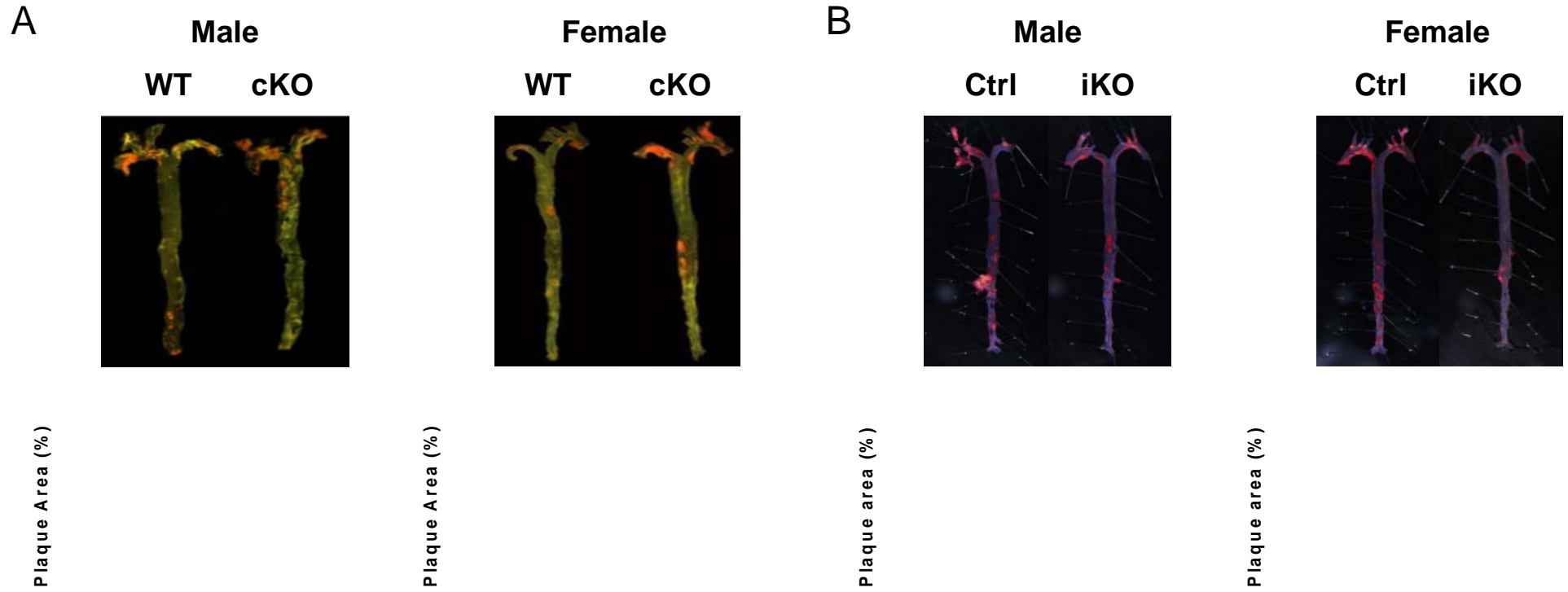
The loss of circadian rhythms in *Bmal1* iKO mice.



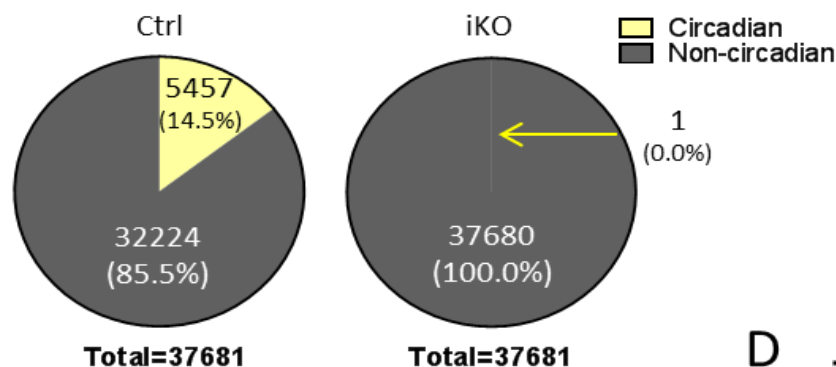
Survival unaltered by postnatal depletion of Bmal1



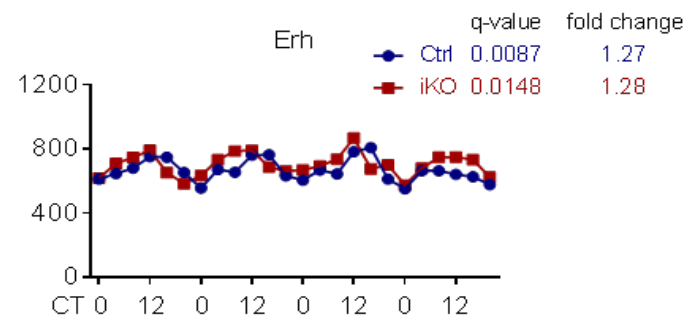
Contrasting phenotypes in cKO and iKO mice.



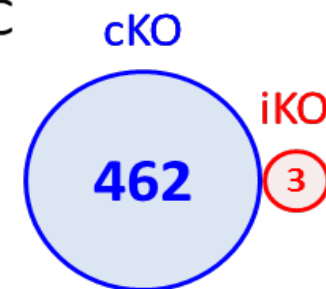
A



B



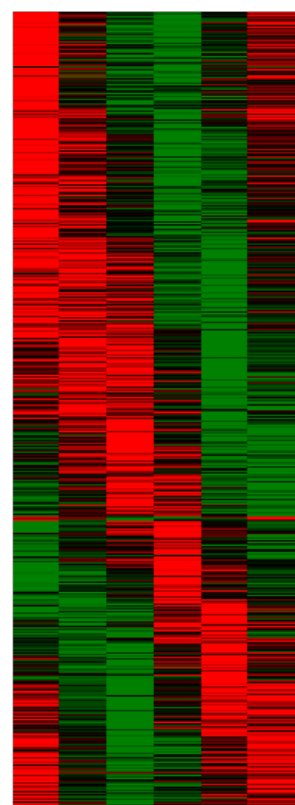
C



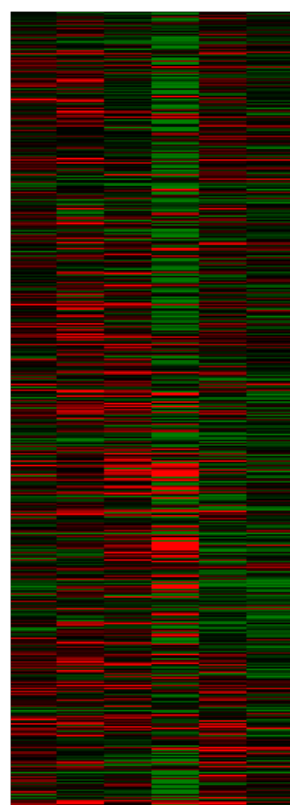
D

Term	Overlap (list/background)	Adjusted P-value
MP0001764 abnormal homeostasis	90/2652	1.47E-12
MP0002139 abnormal hepatobiliary system physiology	31/469	1.74327E-09
MP0002138 abnormal hepatobiliary system morphology	29/619	7.7271E-06
MP0001544 abnormal cardiovascular system physiology	39/1130	8.25166E-05
MP0001657 abnormal induced morbidity/mortality	19/387	0.000314547
MP0002163 abnormal gland morphology	36/1129	0.00071946
MP0002168 other aberrant phenotype	10/135	0.000833642
MP0002060 abnormal skin morphology	24/630	0.000833642
MP0005266 abnormal metabolism	19/437	0.000833642
MP0002127 abnormal cardiovascular system morphology	37/1223	0.000971376
MP0002164 abnormal gland physiology	17/397	0.00171577
MP0010769 abnormal survival	73/3156	0.00171577
MP0002106 abnormal muscle physiology	23/671	0.00316421
MP0005501 abnormal skin physiology	11/211	0.003817395
MP0010678 abnormal skin adnexa morphology	20/582	0.006092002
MP0005164 abnormal response to injury	13/342	0.017562299
MP0000685 abnormal immune system morphology	37/1575	0.047076259

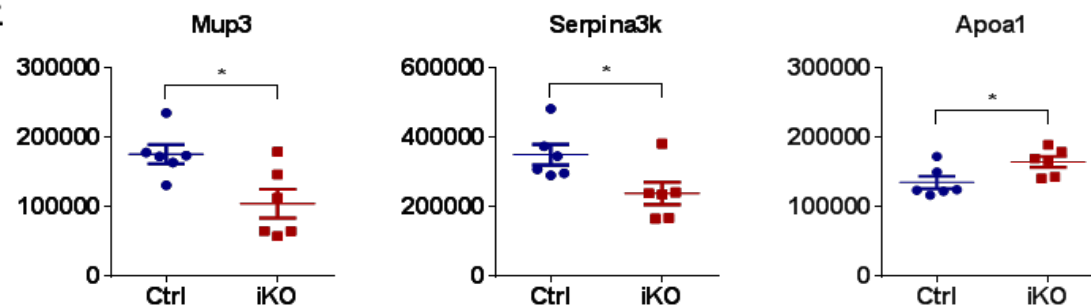
CT 0 4 8 12 16 20



0 4 8 12 16 20

High ■ ■ ■ Low

E

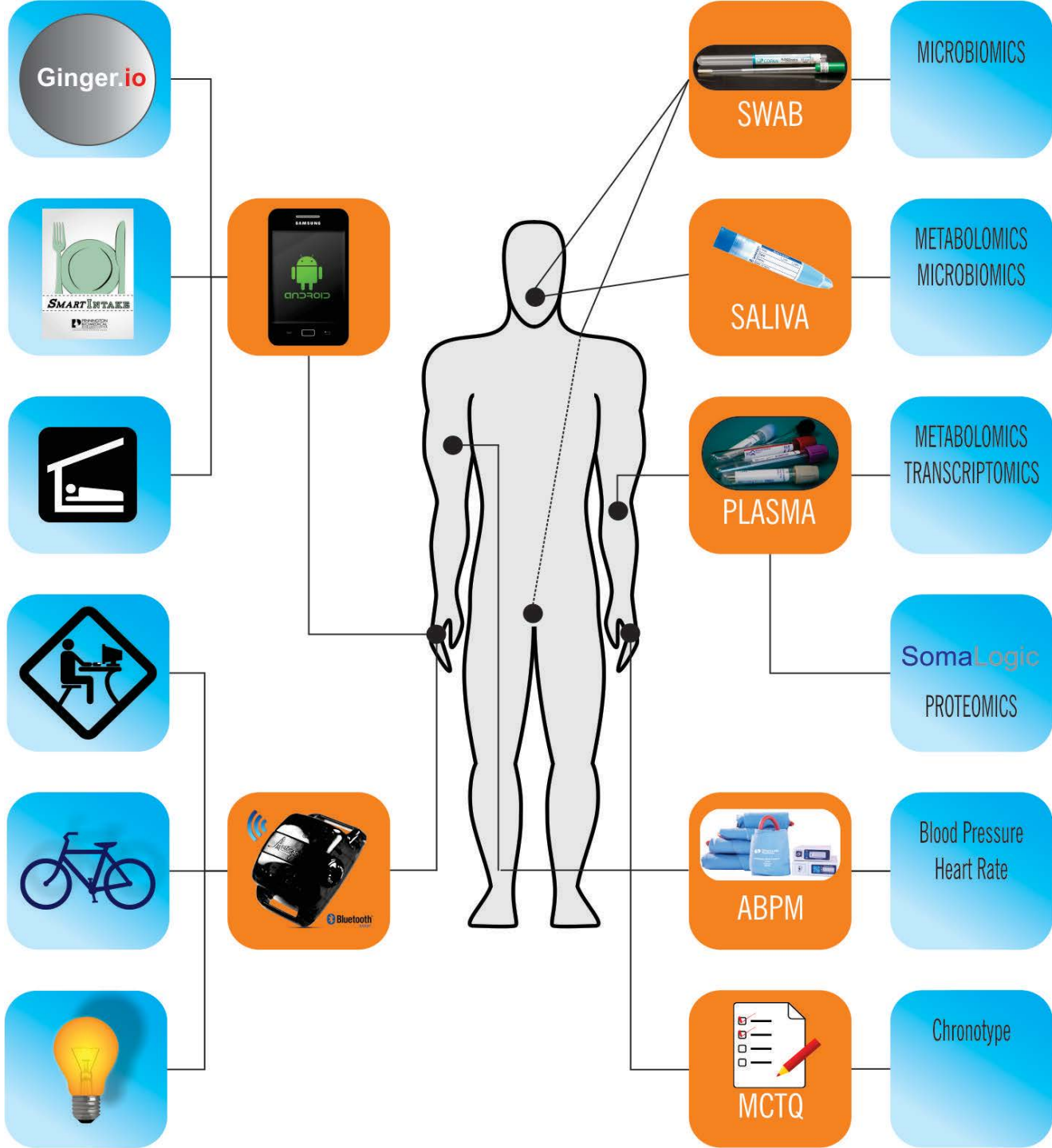


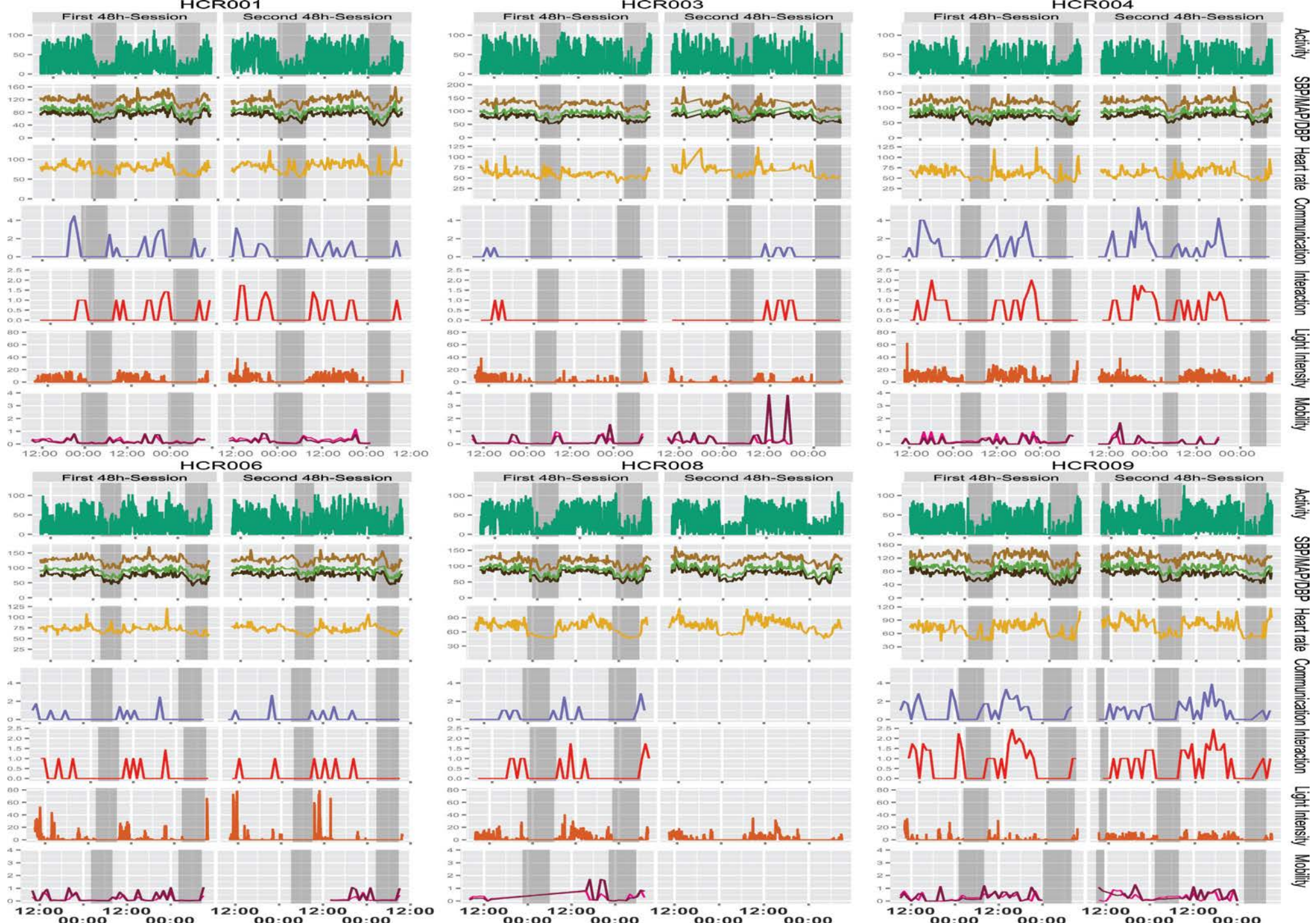
Summary

- Despite similar depletion of clock genes and loss of behavioral and cardiovascular oscillatory phenotypes, survival is markedly different in mice in which Bmal-1 is deleted pre- or postnatally.
- While some aging phenotypes are retained, others are reversed in the iKOs.
- Many more genes dysregulated in the cKOs, suggesting many phenotypes attributed to the clock may reflect off target effects of Bmal-1 during development.

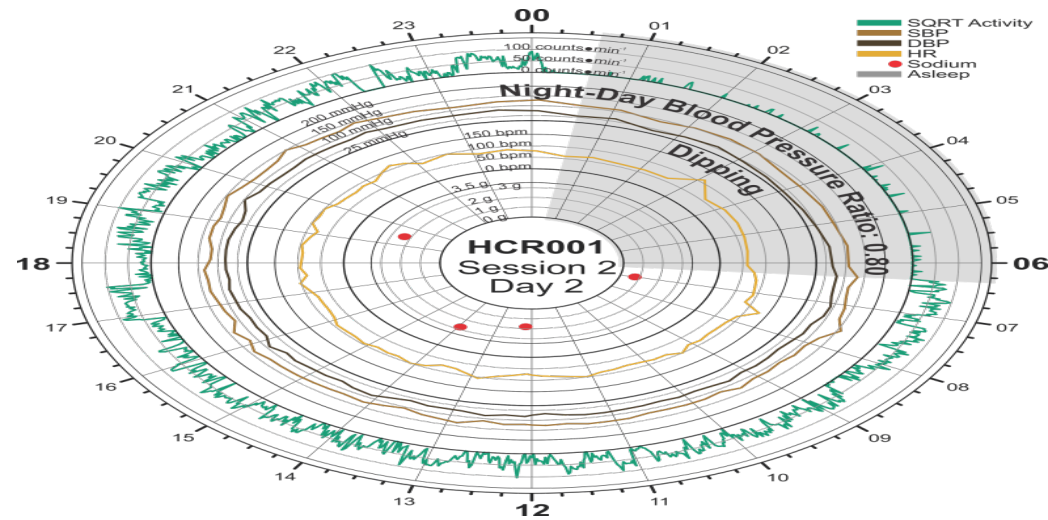
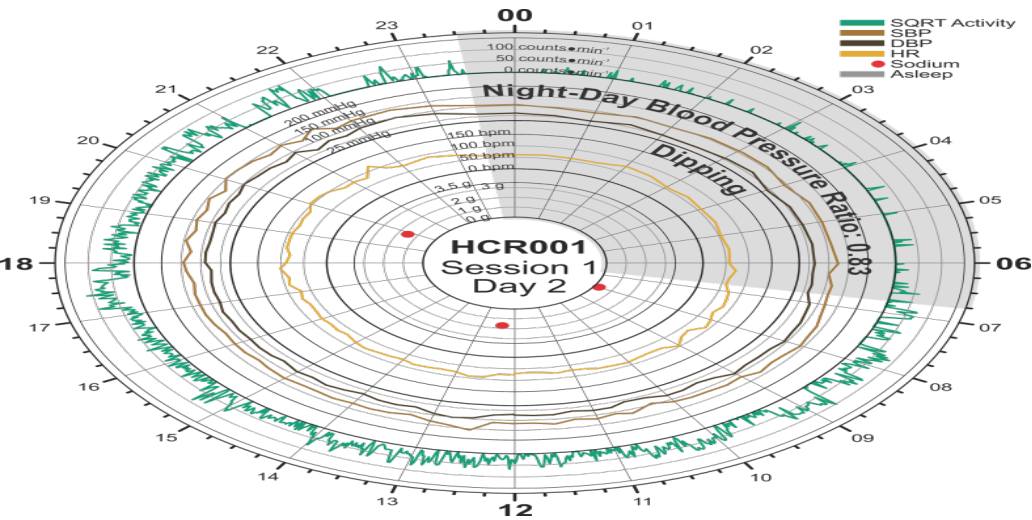
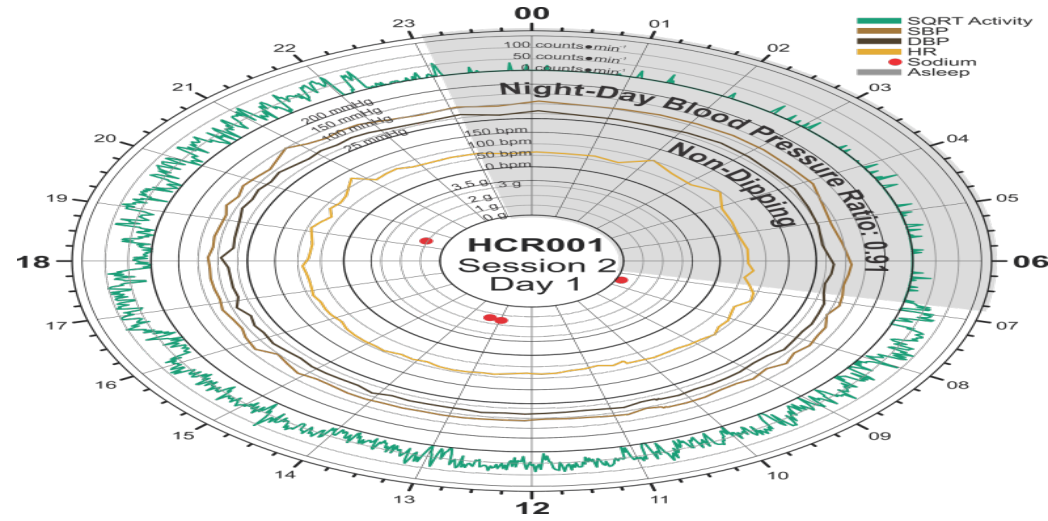
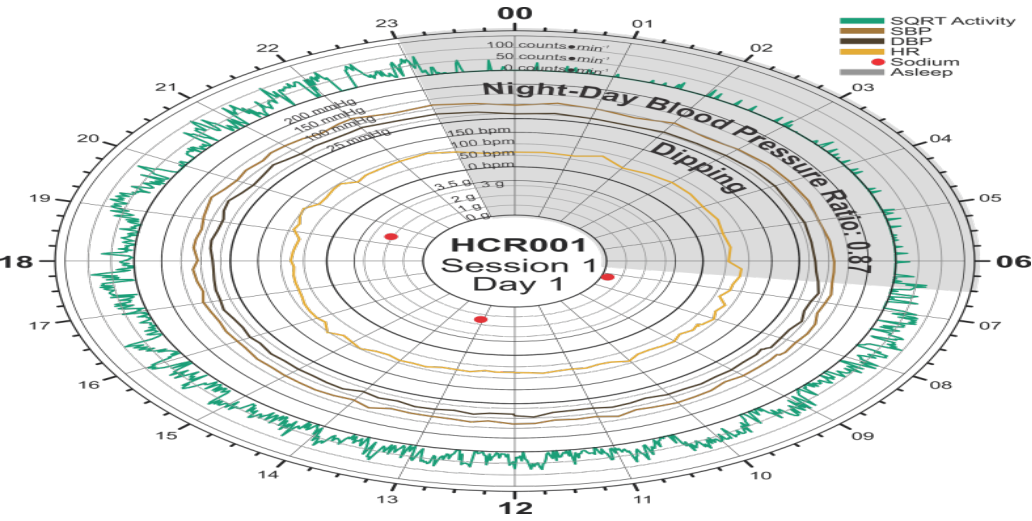
Diurnal Variability in the Human Condition

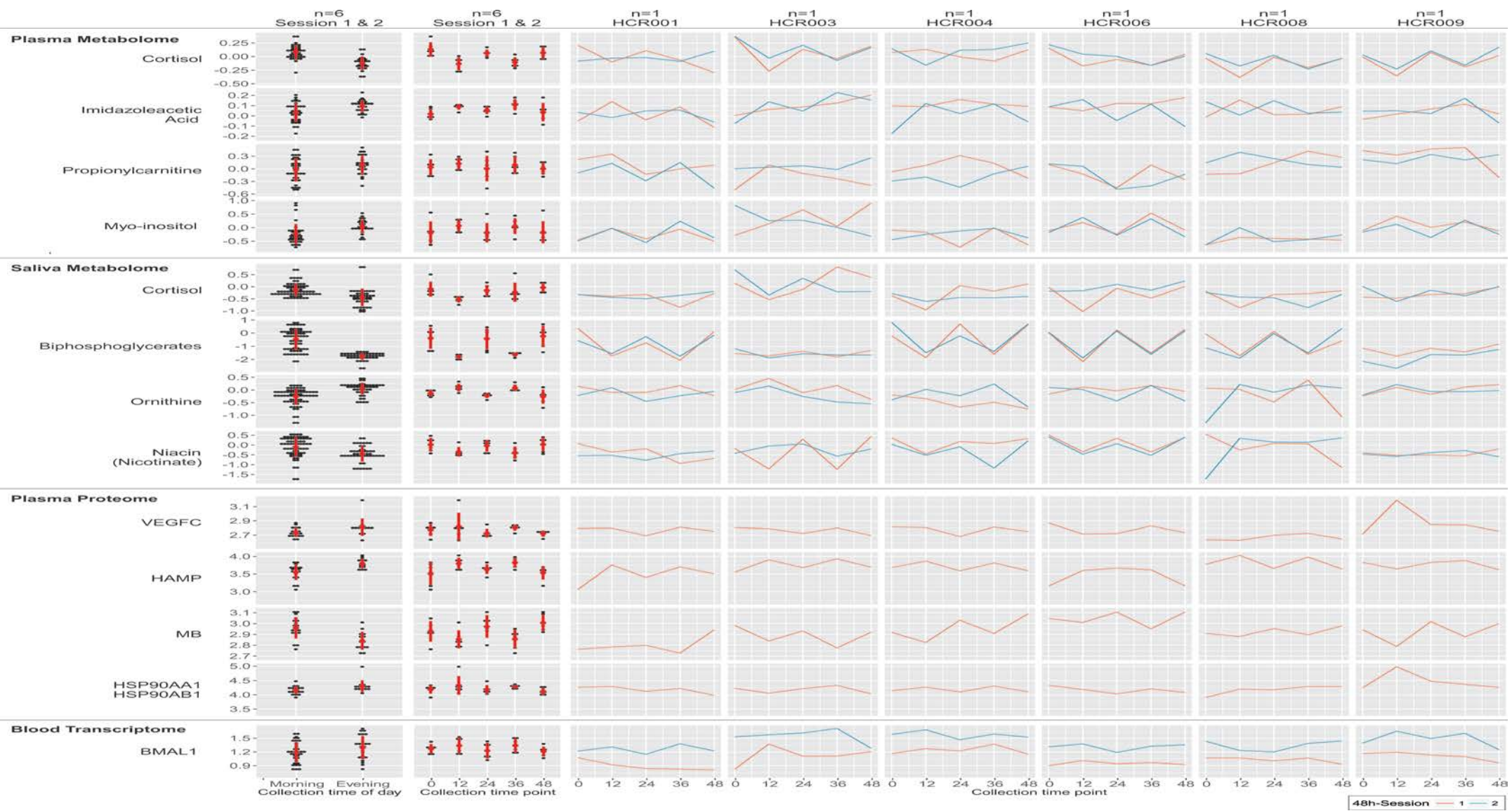
- Heart attack, stroke
- Depression, Suicide and Suicidal attempts
- Asthma
- Arthritis pains
- Memory retrieval, cognitive function
- Exercise preference
- Eating and drinking behavior





HCR001

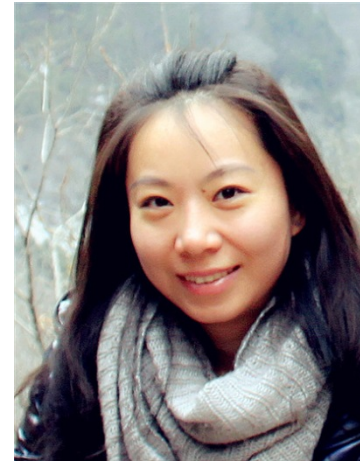




The Human Chronobiome

- Integration of omics and remote sensing approaches to determine signal:noise detection in humans “in the wild”.
- Determination of variance, integration of heterogeneous data and examination of age and sex as variables.
- Basal and evoked phenotypes in healthy individuals a basis for seeking divergence in time dependent expression of disease.

Guangrui, Xue and Carsten



Truth is the daughter of time and I
feel no shame in being her
midwife

Johannes Kepler