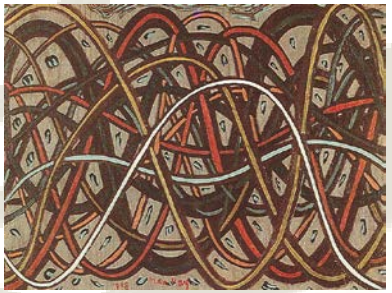


Circadian Control of Xenobiotic Carcinogenesis and Anticancer Drug Pharmacology

Francis Lévi

Warwick Medical School

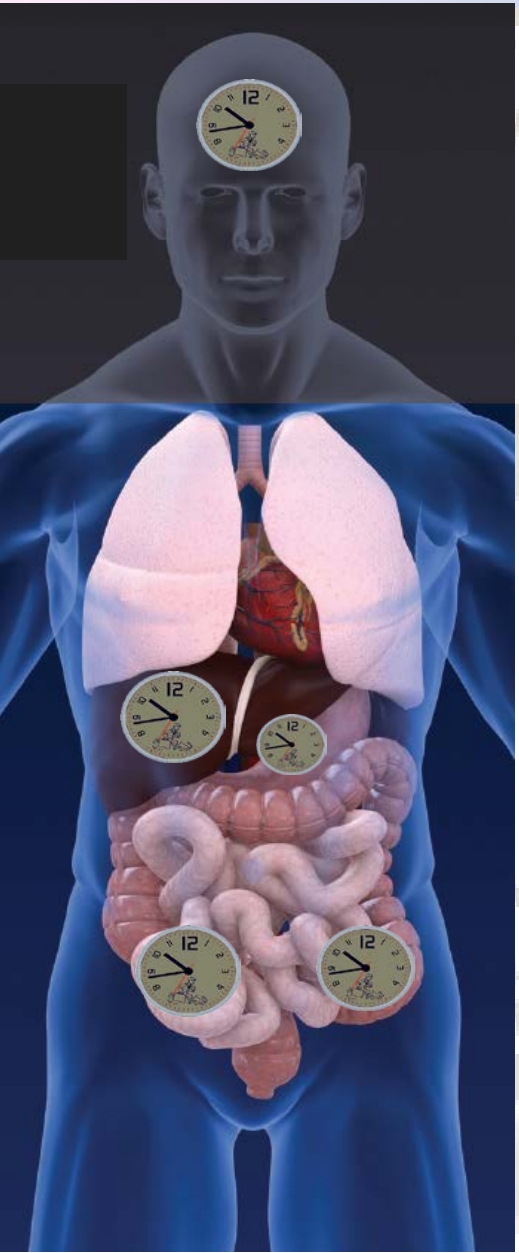
Cancer Chronotherapy Unit
Cancer Research Unit

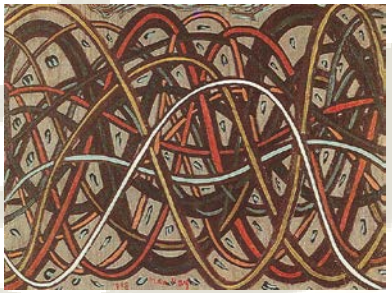
Rythmes Biologiques et Cancers UMR S776

Paul Brousse hospital, Villejuif (France)

Inserm

UNIVERSITÉ PARIS SUD





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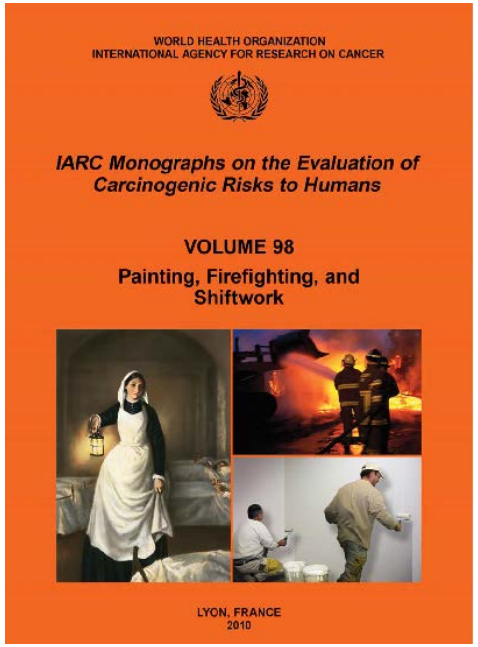
Carcinogenicity of shift-work, painting, and fire-fighting

Kurt Straif, Robert Baan, Yann Grosse, Béatrice Secretan, Fatiha El Ghissassi, Véronique Bouvard, Andrea Altieri, Lamia Benbrahim-Tallaa, Vincent Cogliano, on behalf of the WHO International Agency for Research on Cancer Monograph Working Group

WORLD HEALTH ORGANIZATION
INTERNATIONAL AGENCY FOR RESEARCH ON CANCER



On the basis of "limited evidence in humans for the carcinogenicity of shift-work that involves nightwork", and "sufficient evidence in experimental animals for the carcinogenicity of light during the daily dark period (biological night)",

the Working Group concluded that

"shift-work that involves circadian disruption is probably carcinogenic to humans" (Group 2A)

Lancet Oncology 2007; IARC Monograph #98, 2010, 804 pp.

The ten hallmarks of cancer

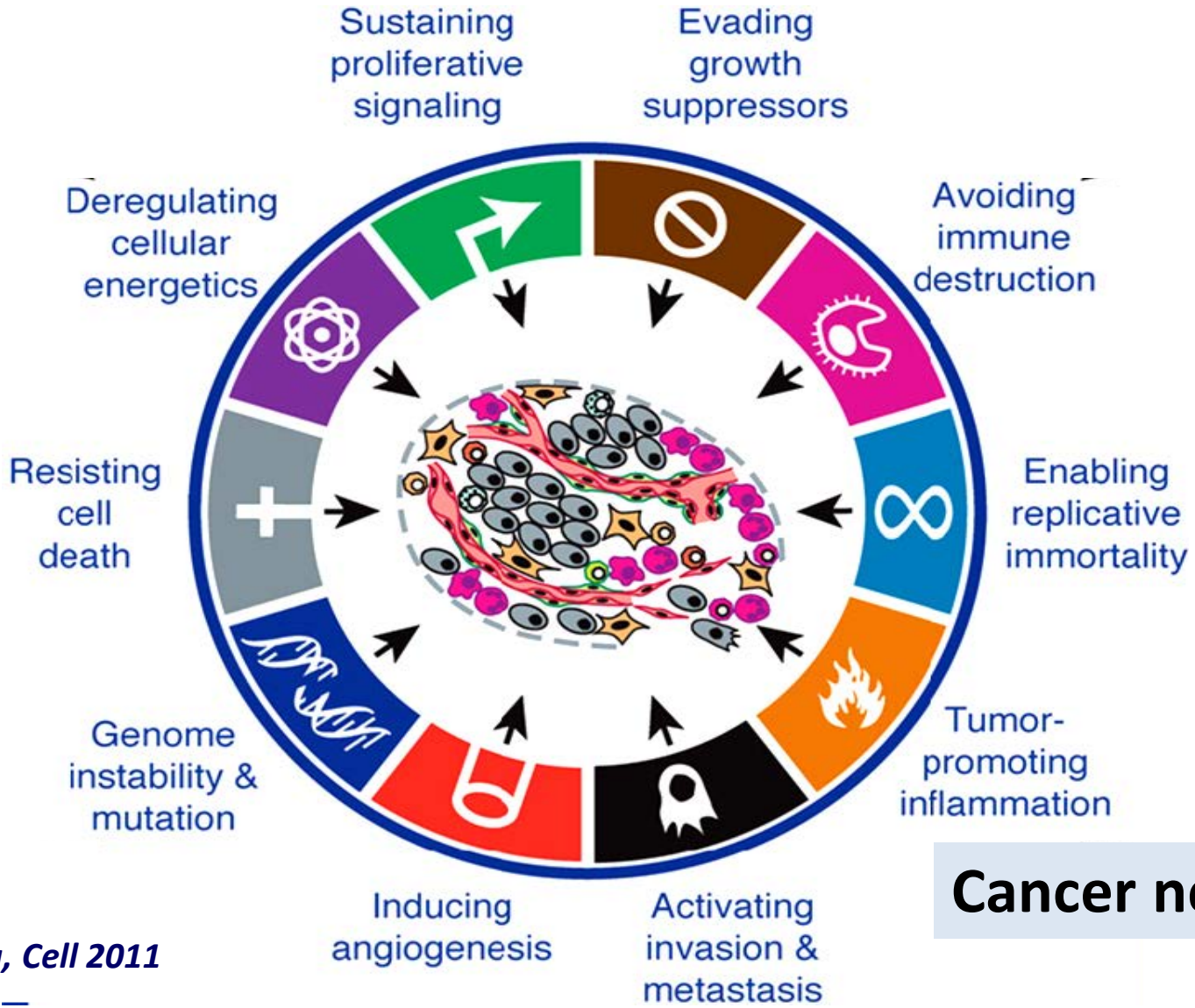
Cancer

Cancer cells

Leukocytes

Dendritic cellls

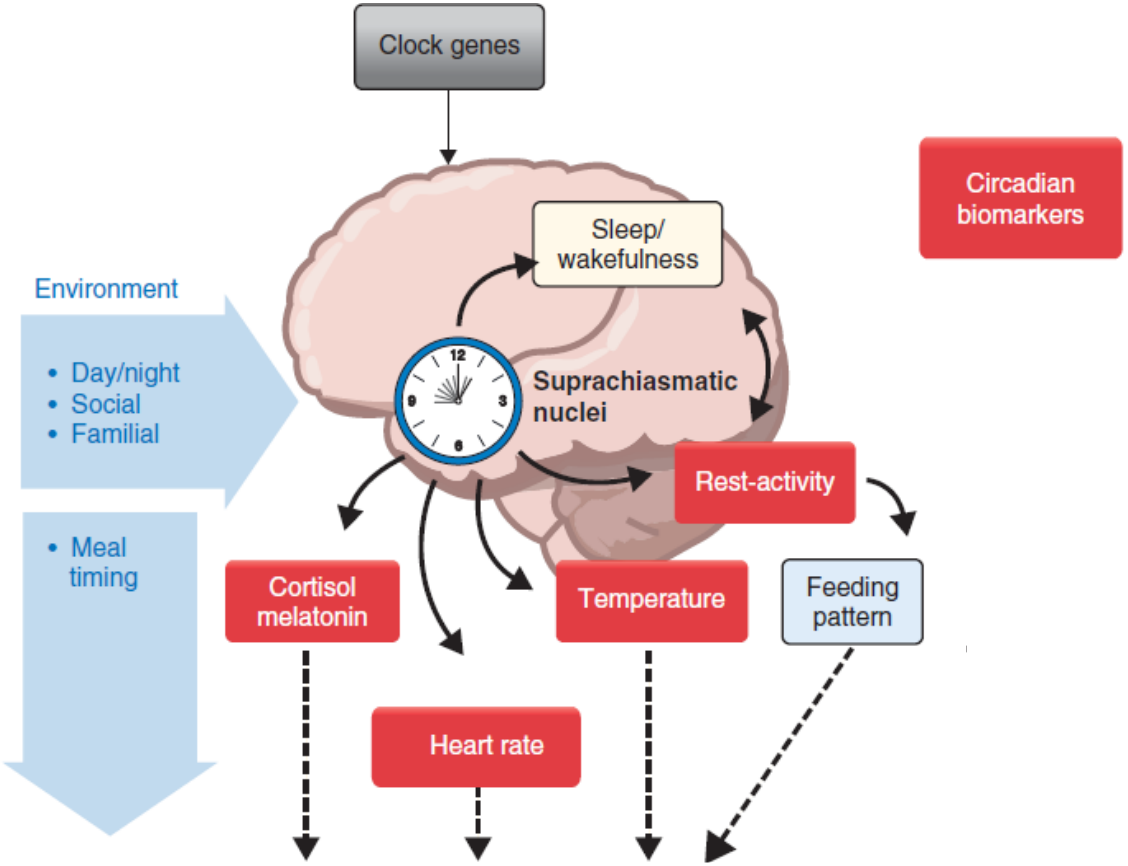
Blood vessels



Cancer needs ≥ 6

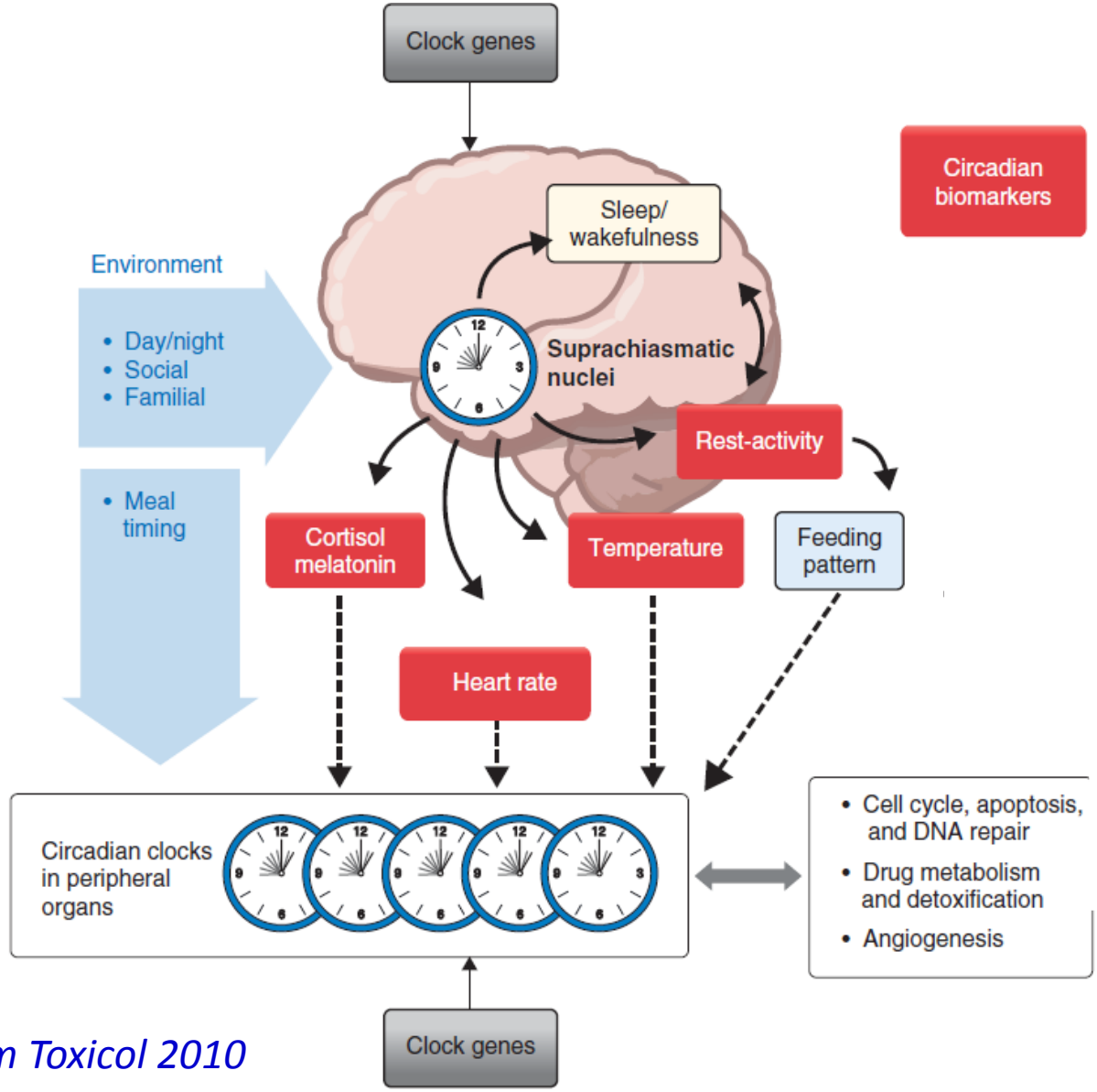
Hanahan and Weinberg, Cell 2011

The Circadian Timing System



Lévi et al. Annu Rev Pharm Toxicol 2010
Lévi & Okyar EODD 2011

The Circadian Timing System



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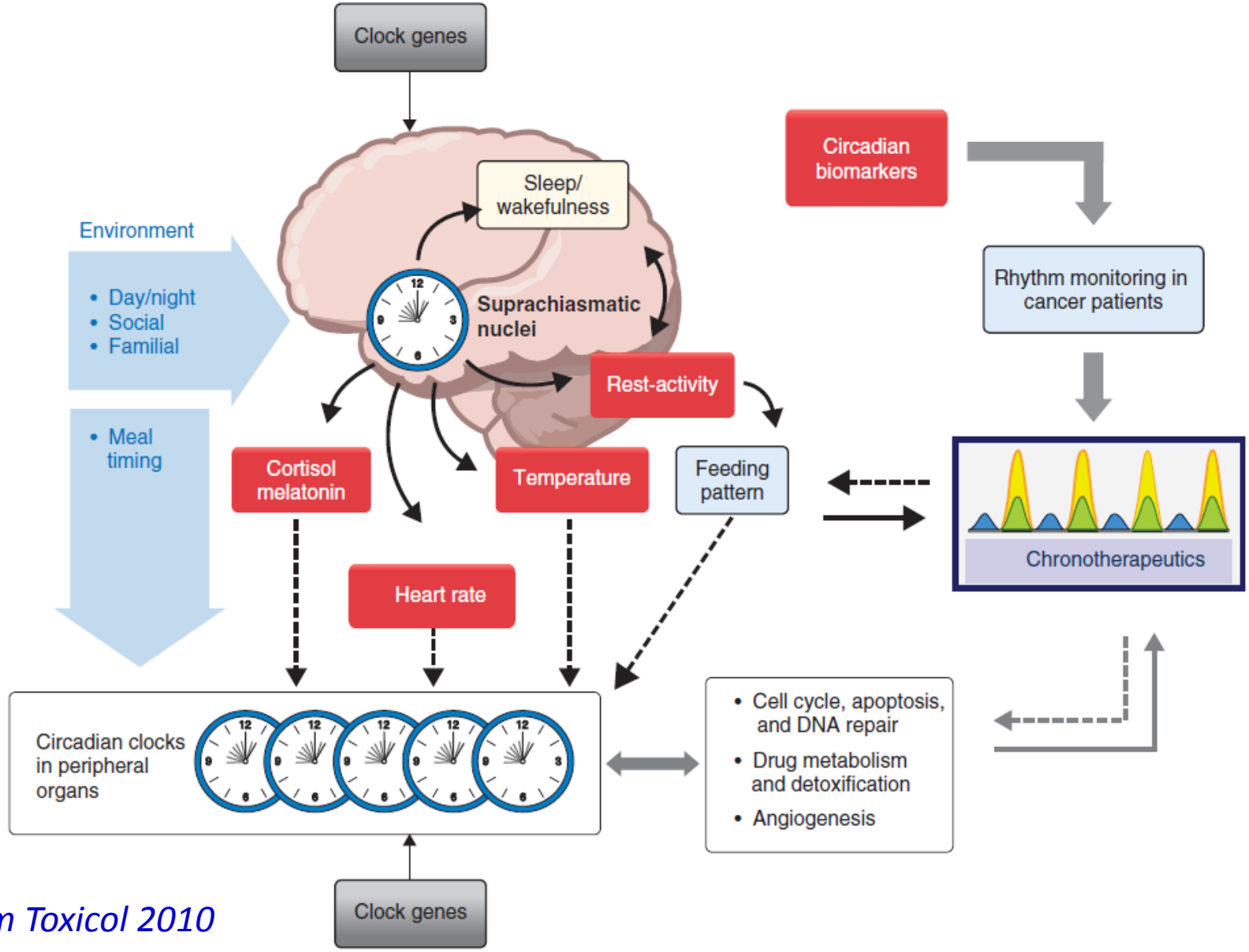

Lévi et al. Annu Rev Pharm Toxicol 2010
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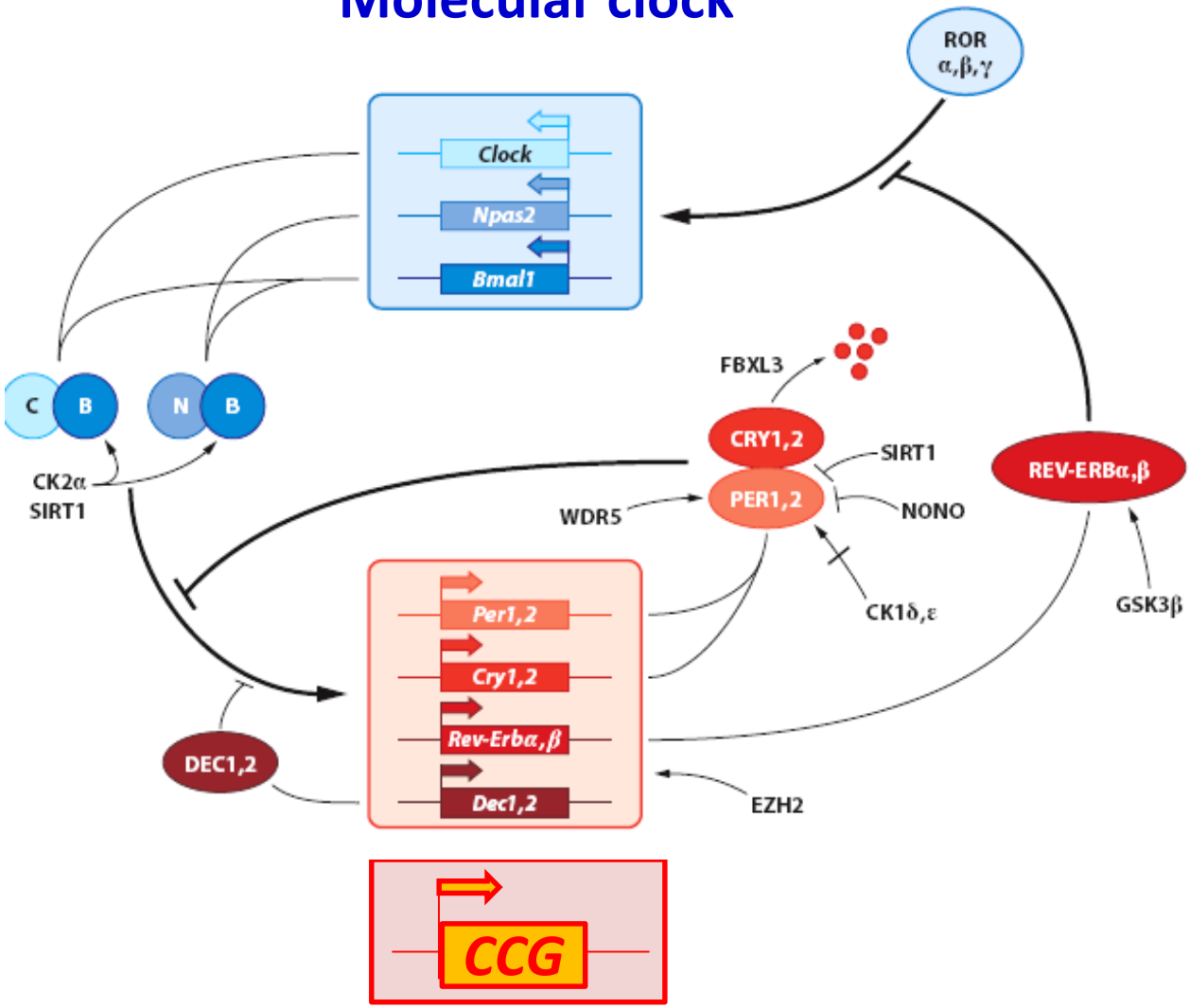
Molecular clock



Lévi et al. Annu Rev Pharm Toxicol 2010

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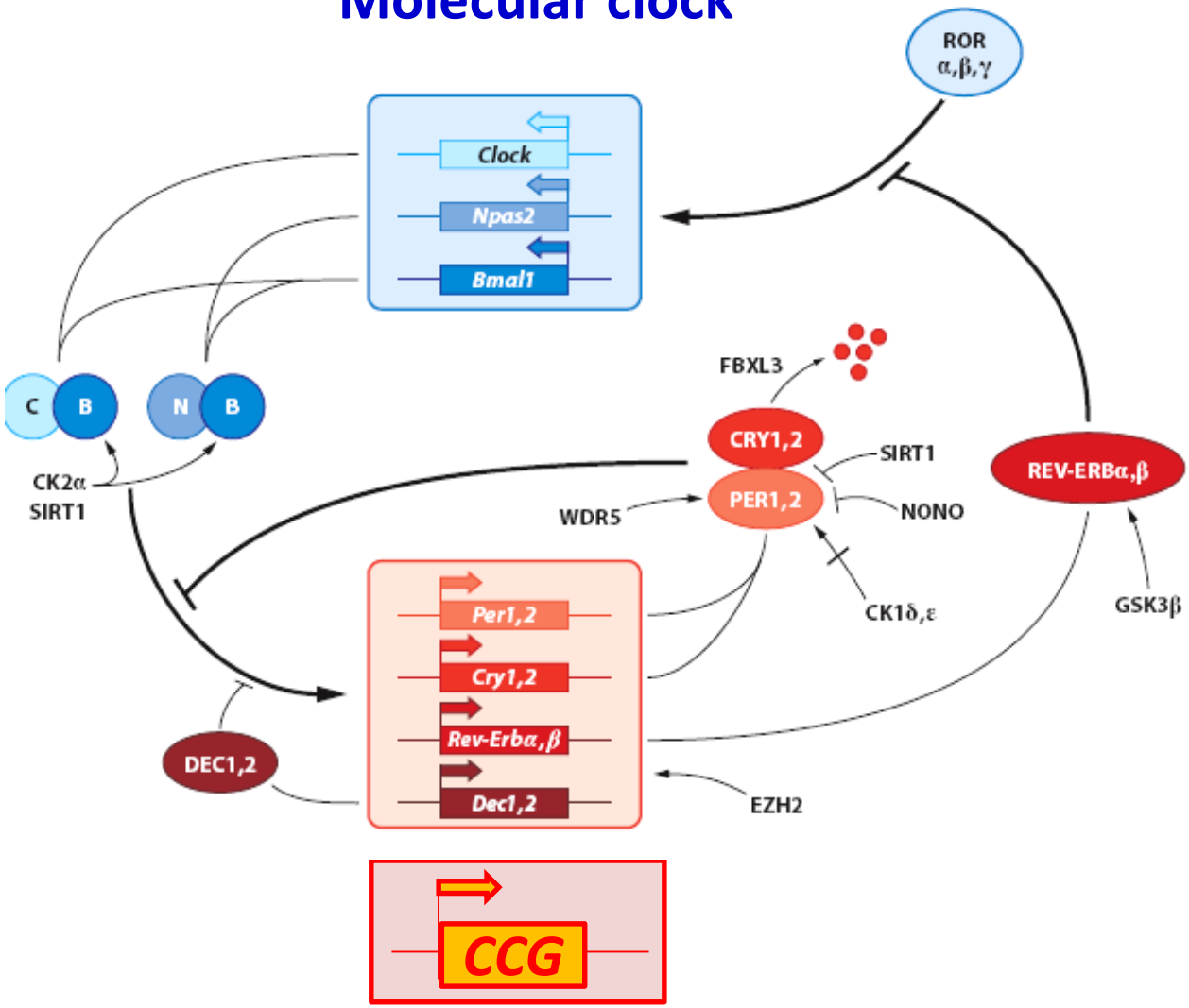
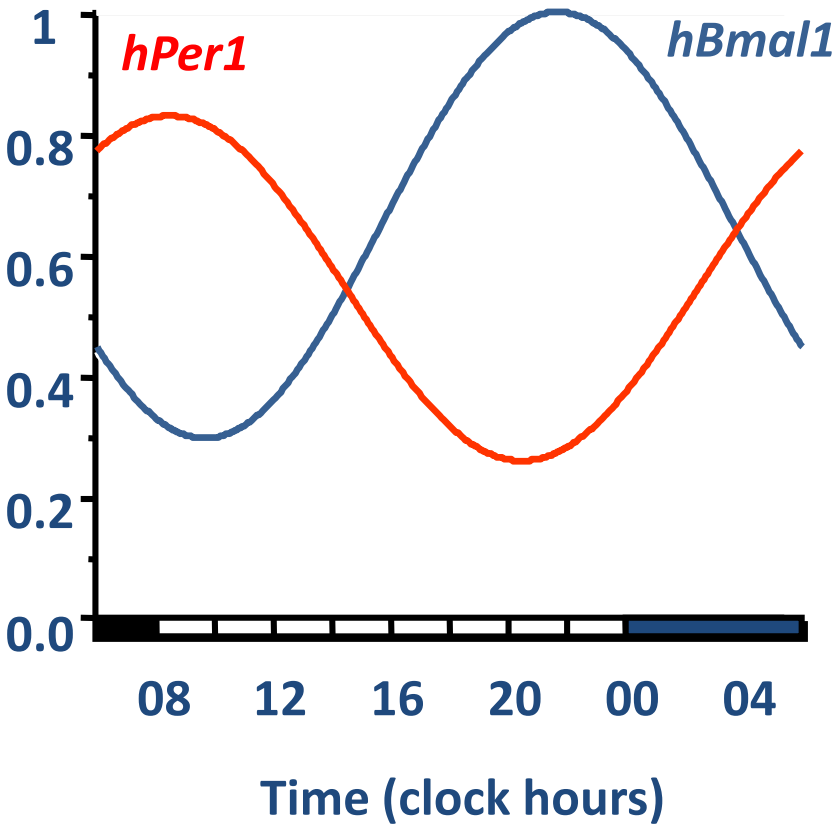


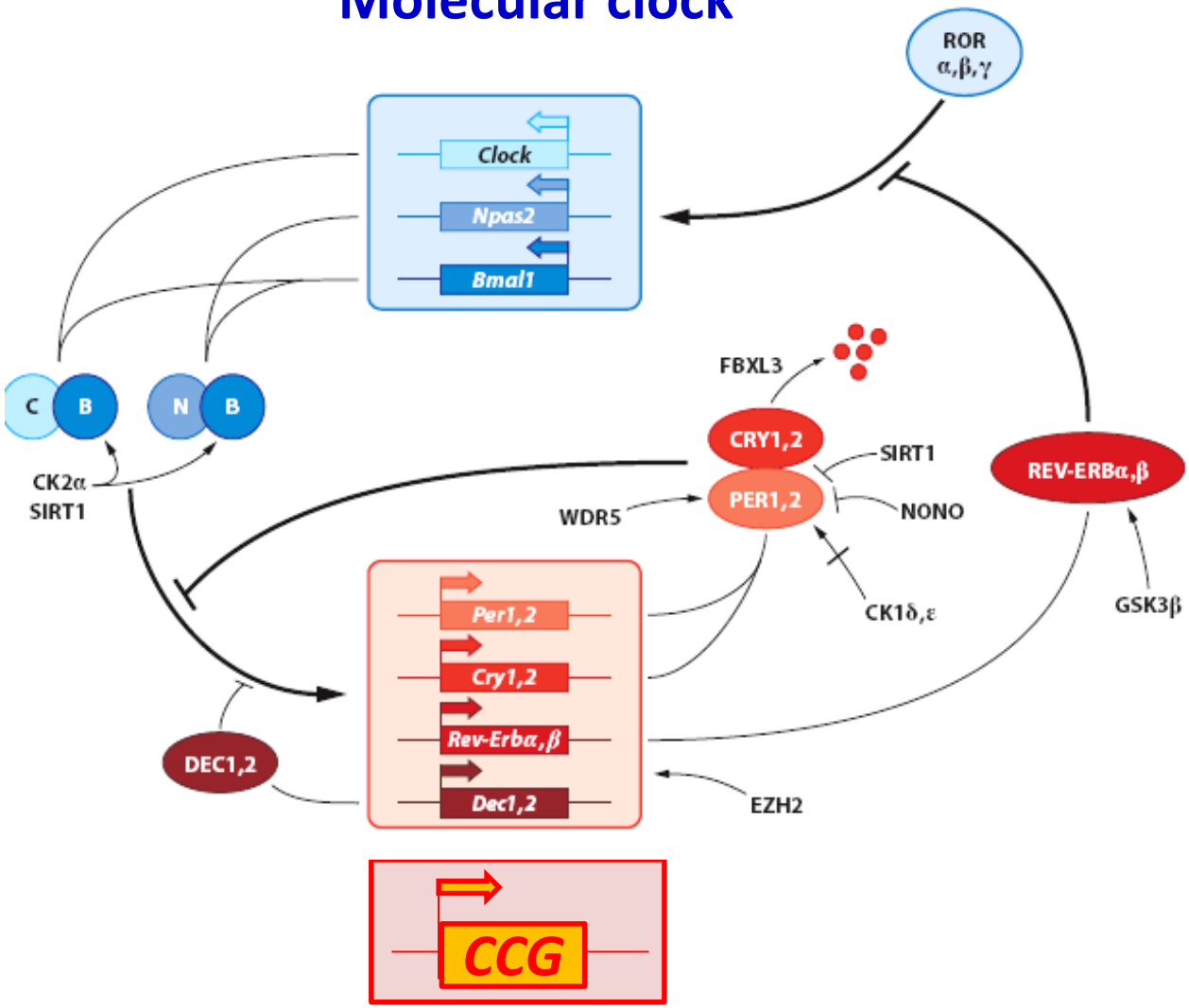
Lévi et al. Annu Rev Pharm Toxicol 2010

Clock gene transcription rhythms in human oral mucosa



Bjarnason et al. Am. J Pathology 2001

Molecular clock



Lévi et al. Annu Rev Pharm Toxicol 2010

Clock-Controlled genes

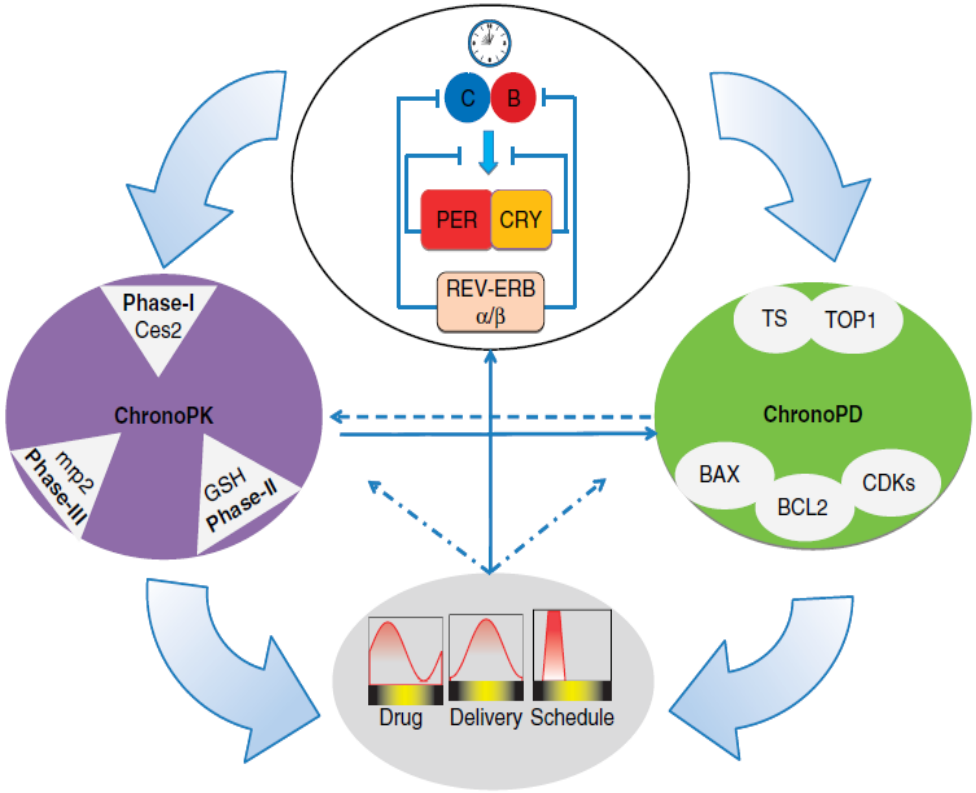
- **Drug metabolism and detoxification**
  Cyp3a, Ces1-3, UGT1A1, GST-π, Upa, Dpyd,…

- **Drug transport**
  Abcb1a/b, Abcc2, Abcg2,…

- **Drug targets**
  TS, Top1, Top2, mTOR…

- **Cell cycle, apoptosis, repair**
  Wee1, P21, P53, c-Myc, Bcl-2, Bax, Mdm2, cyclin D, Tip60,…

Circadian Control of Cancerogenesis & Cancer Chronopharmacology



Lévi & Schibler, Annu Rev Pharm Toxicol 2007
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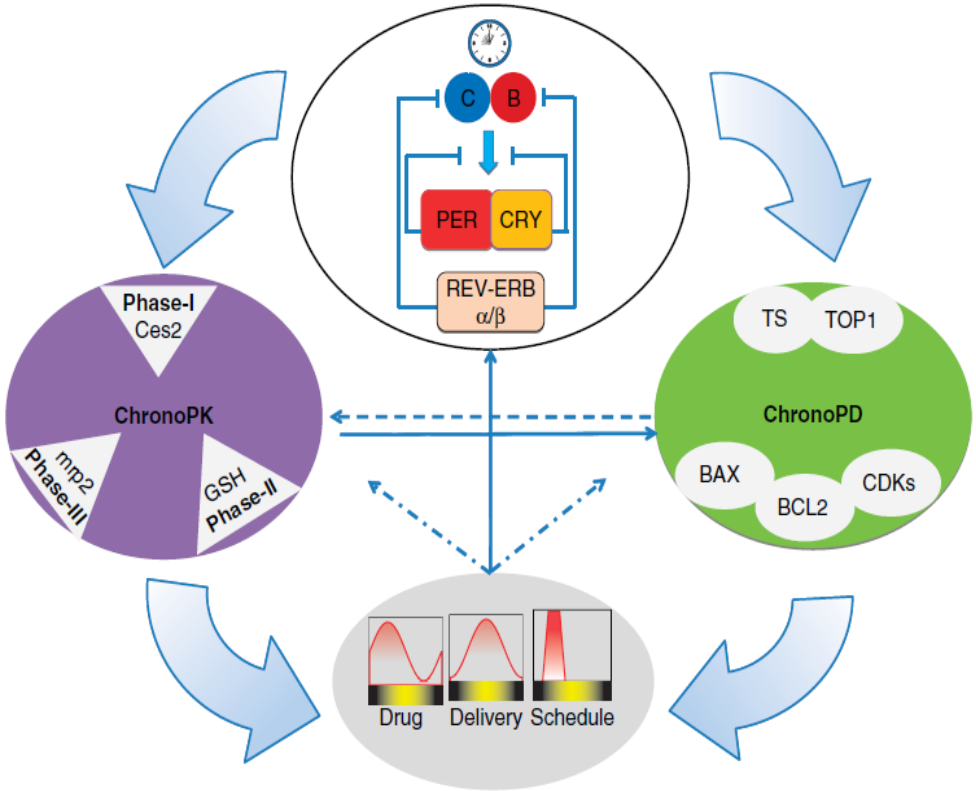

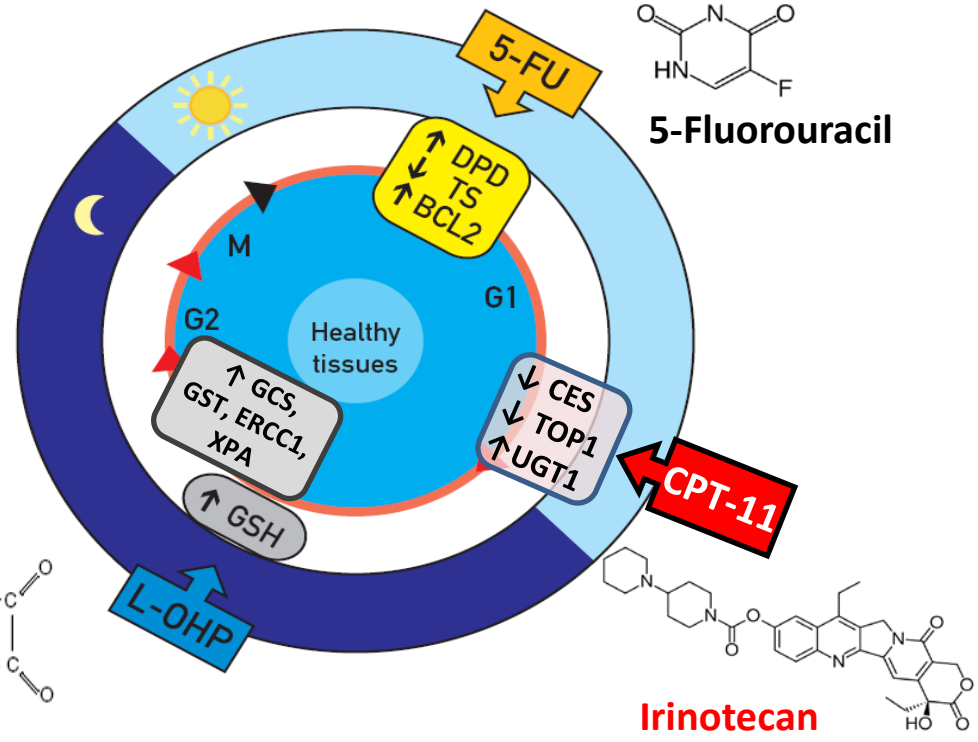

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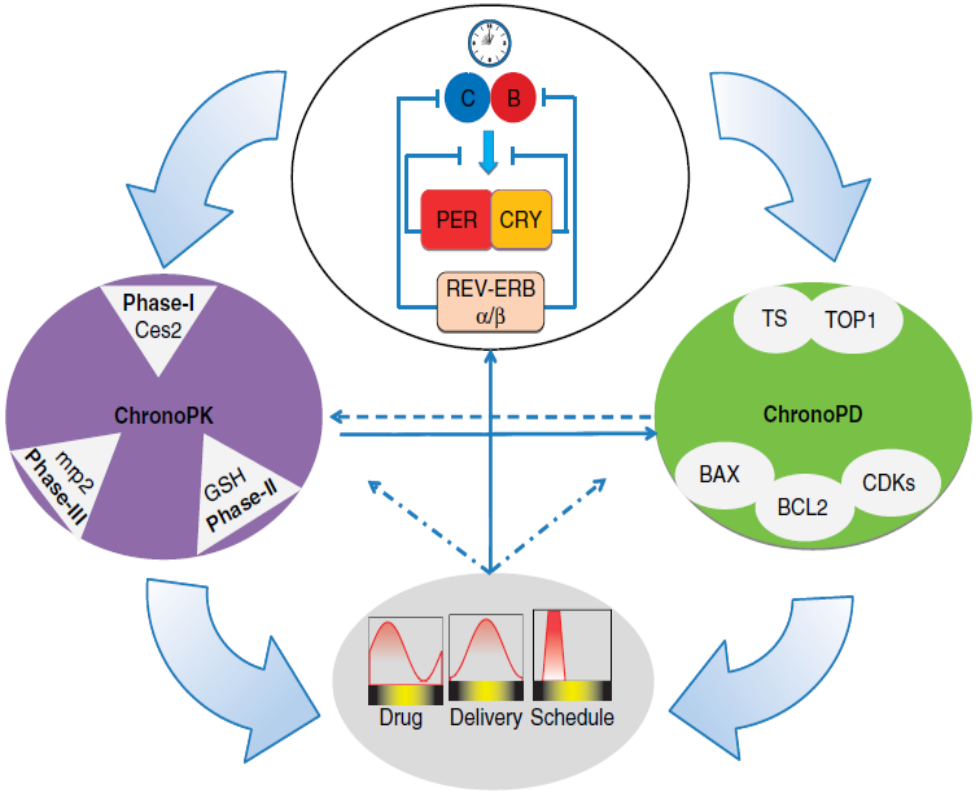

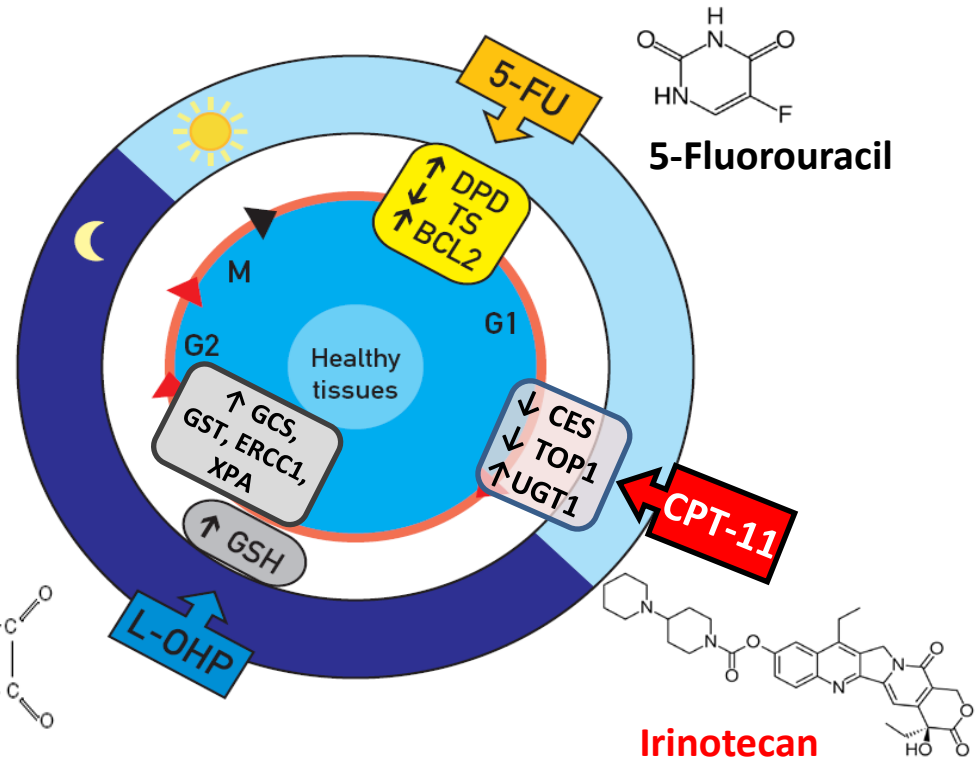

Chronotolerance
40 anticancer drugs
Chronoefficacy
28 anticancer drugs

Lévi & Schibler, Annu Rev Pharm Toxicol 2007
Lévi et al. Annu Rev Pharm Toxicol 2010

Circadian Timing System ↔ Cancer Progression

at Whole Organism level

CTS disruption	Experimental cancer progression
• Suprachiasmatic ablation • Chronic jet lag • ClockΔ19 mutation	→ Two- to three-fold acceleration

CTS re-inforcement	
• Meal Timing	→ Reduced by half to two-third

Filipski et al. J Natl Cancer Inst 2002, 2005; Cancer Res 2004; Mut Res 2009
Wu et al. Life Sci 2004; Li et al. Cancer Res 2010

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CTS disruption on liver cancerogenesis?

- **Cancer promotion**

Rat DEN model
CJL Mouse DEN model

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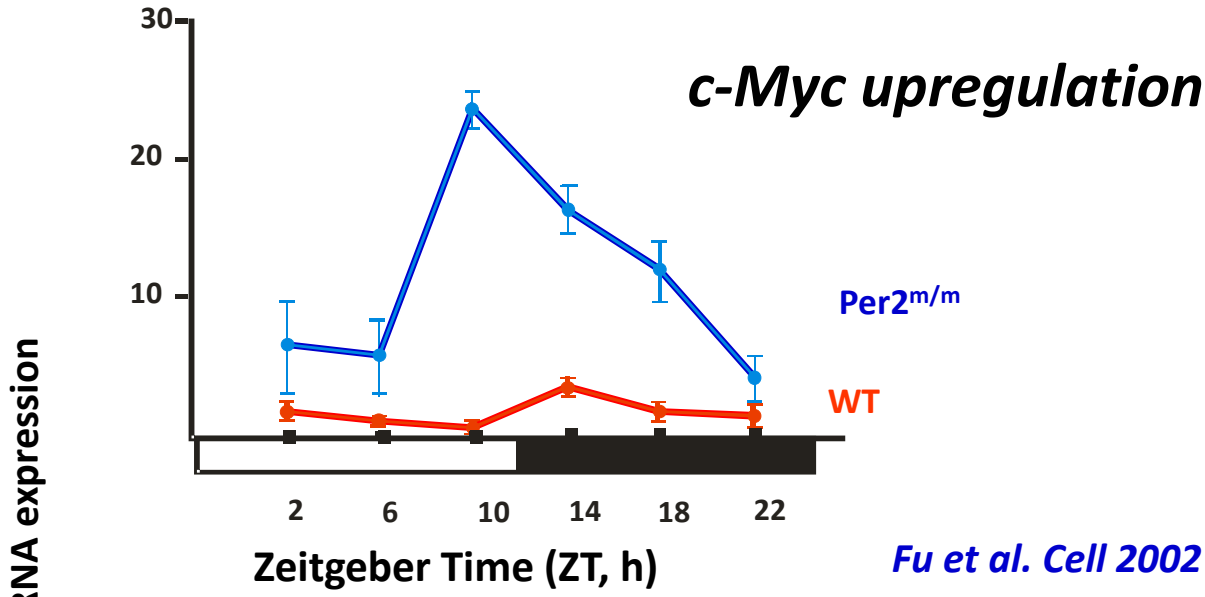
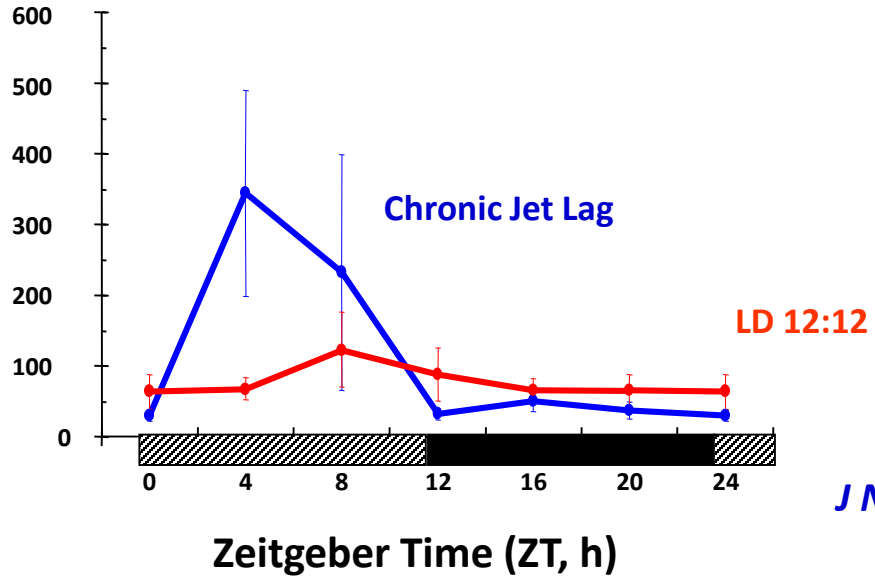
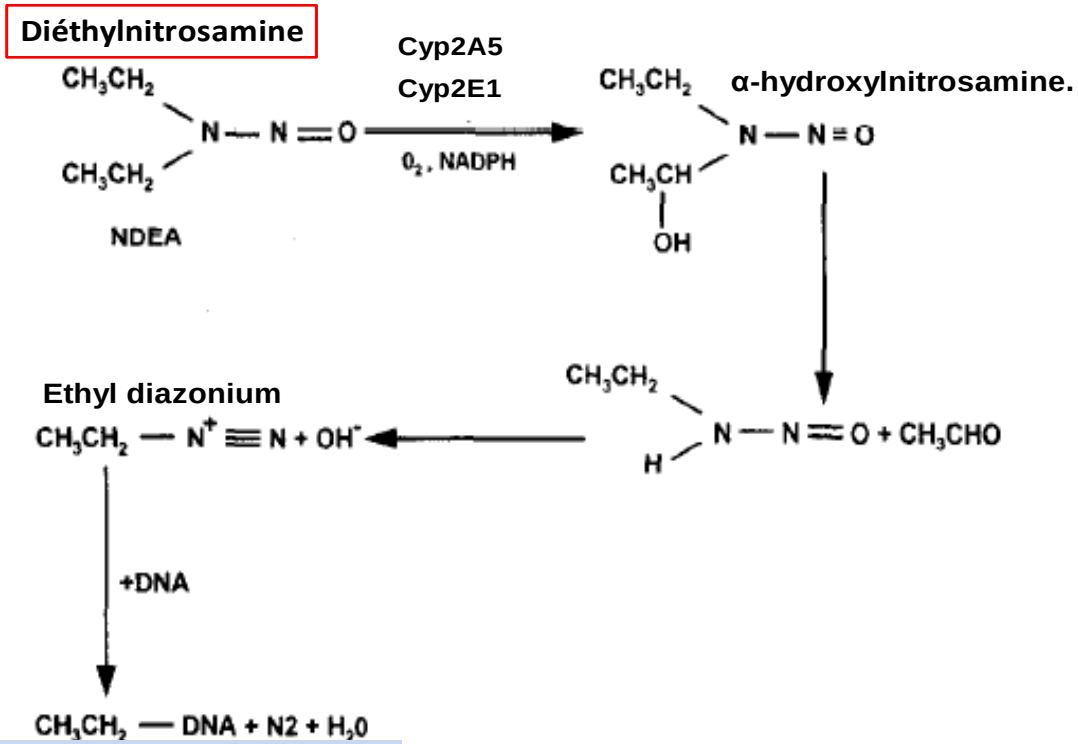


Fu et al. Cell 2002



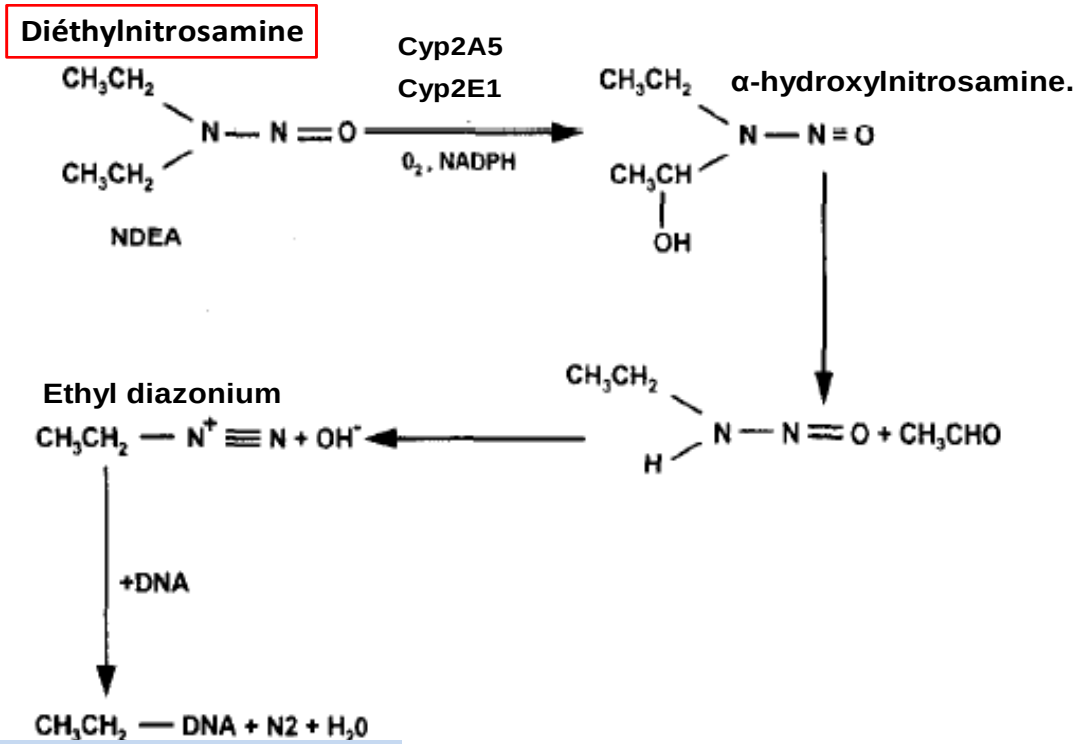
Filipski et al.
J Natl Cancer Inst 2005

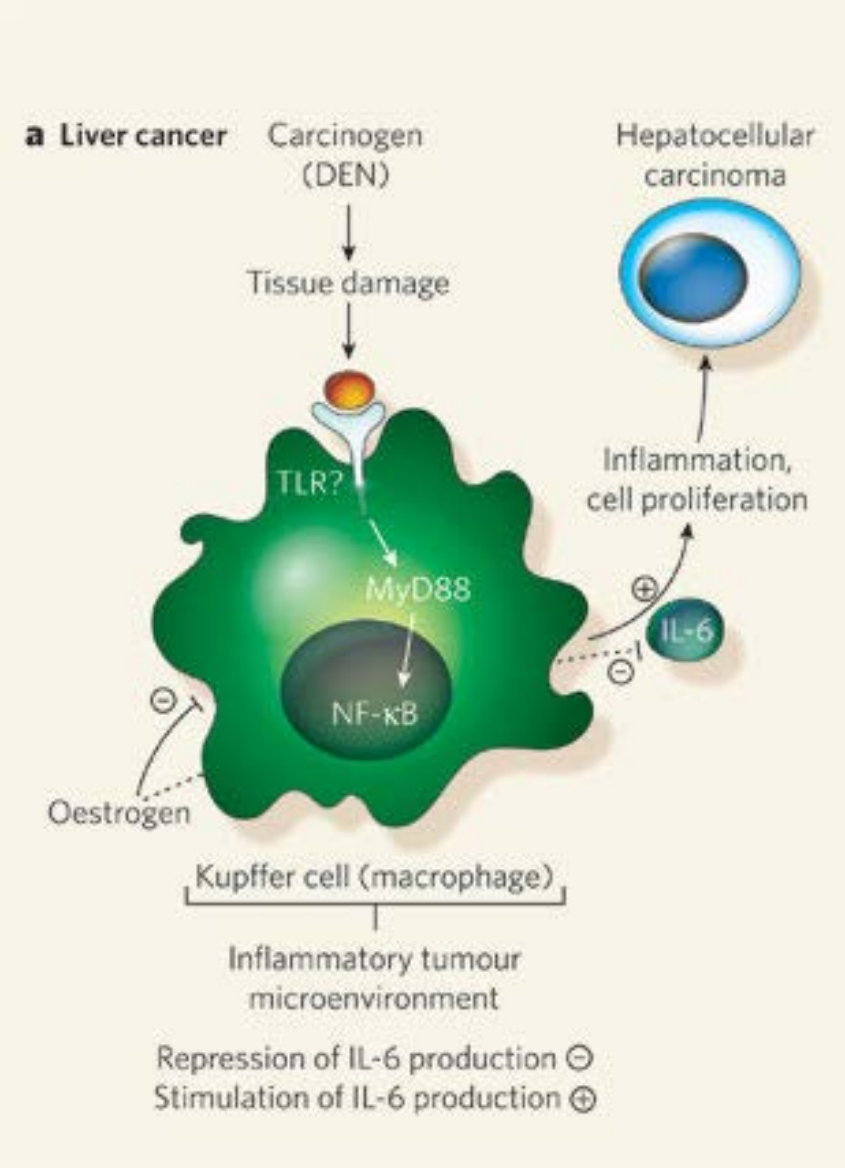
The DEN experimental cancer model



DNA adducts

The DEN experimental cancer model





Circadian Control of Cancerogenesis & Cancer Chronopharmacology

Ali Mteyrek

	Study I		Study II	
	Wt	*Per2*$^{m/m}$	Wt	Cry1$^{-/-}$Cry2$^{-/-}$
Controls	2*	2	2	2
DEN**	13	13	16	15

**N of mice*

***DEN (5 d a week over 50 d at ZT11) = 402 mg/kg*

Elisabeth Filipski

Mteyrek et al. submitted

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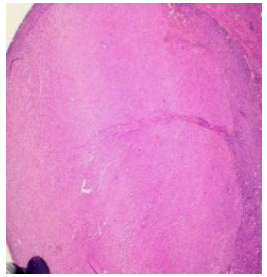
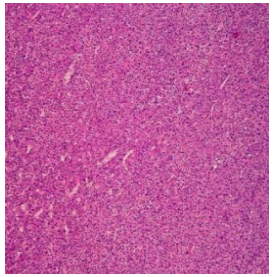
Elisabeth Filipski

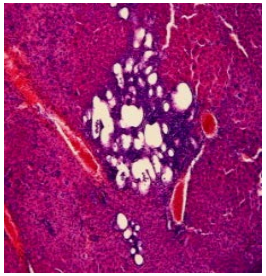
4 months

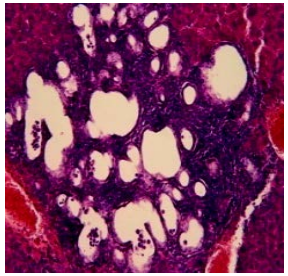
Hepatocarcinoma



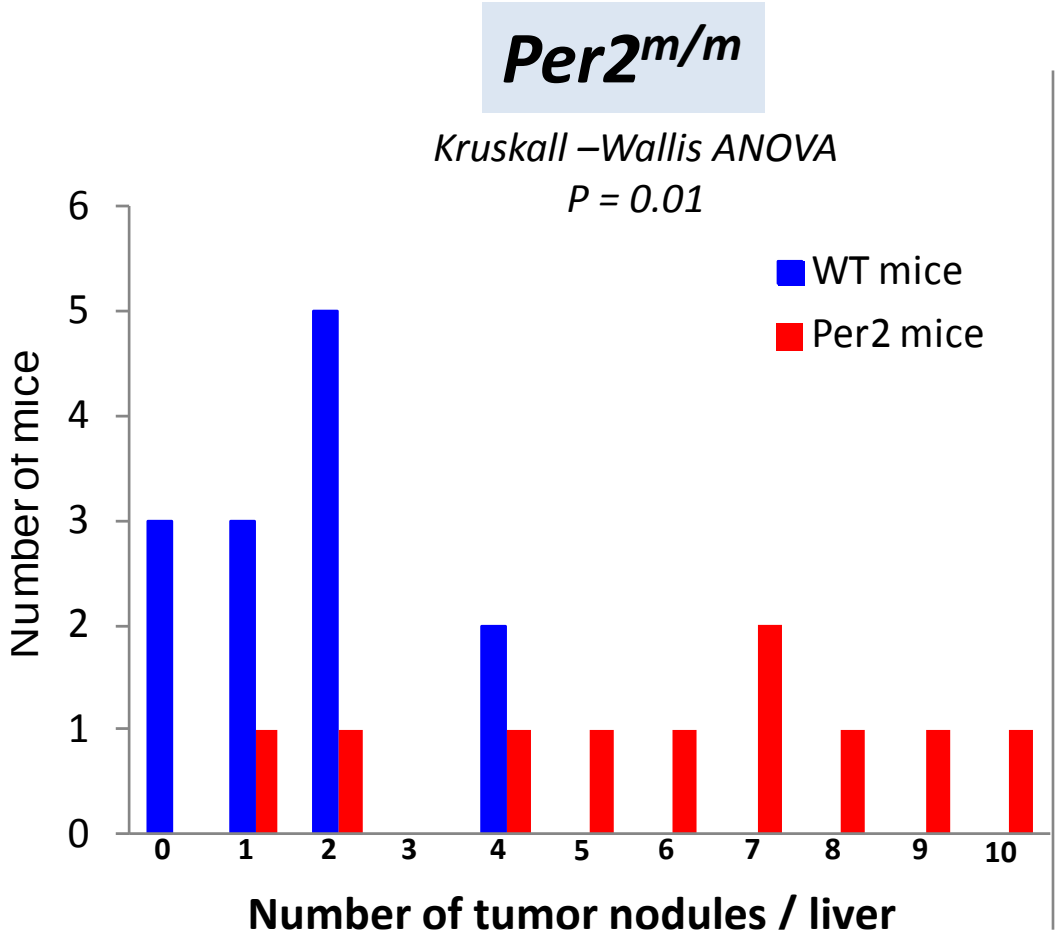


Cholangiocarcinoma





Mteyrek et al. submitted



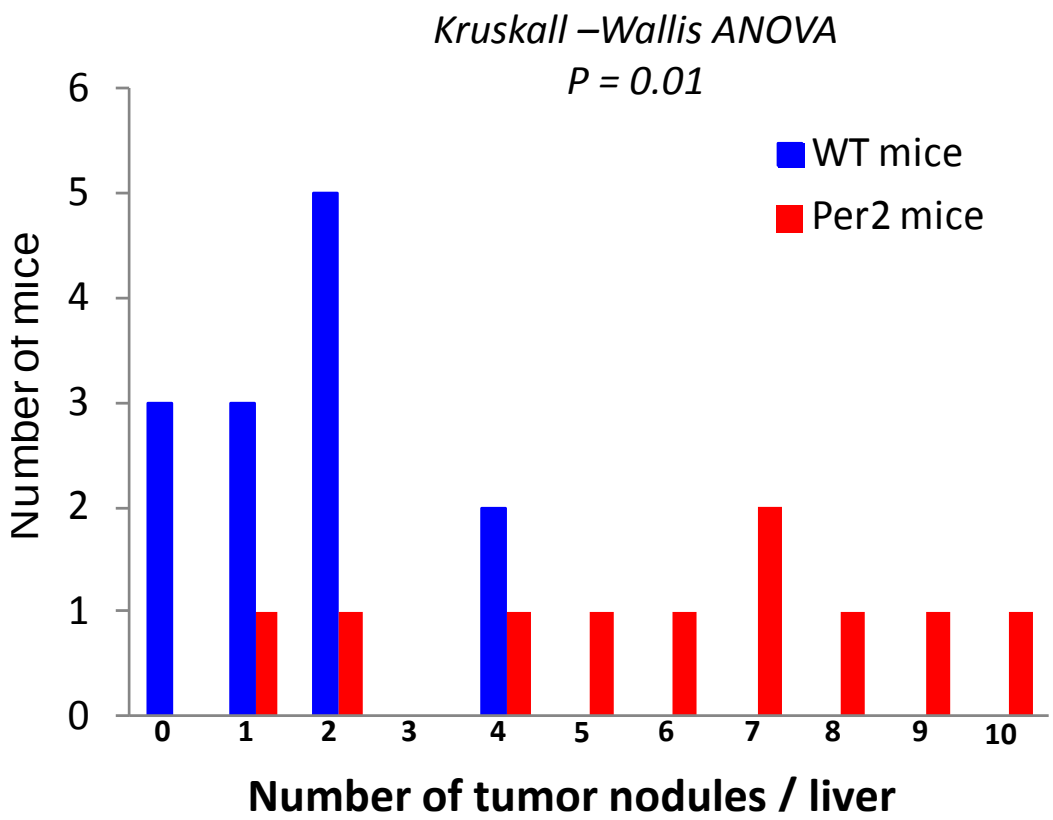
N of mice with >2 nodules

***Wt* : 2/13 (15 %) ; *Per2m/m*: 8/10 (80 %)**

Exact Fisher, p= 0.003

Mteyrek et al. submitted

Per2$^{m/m}$



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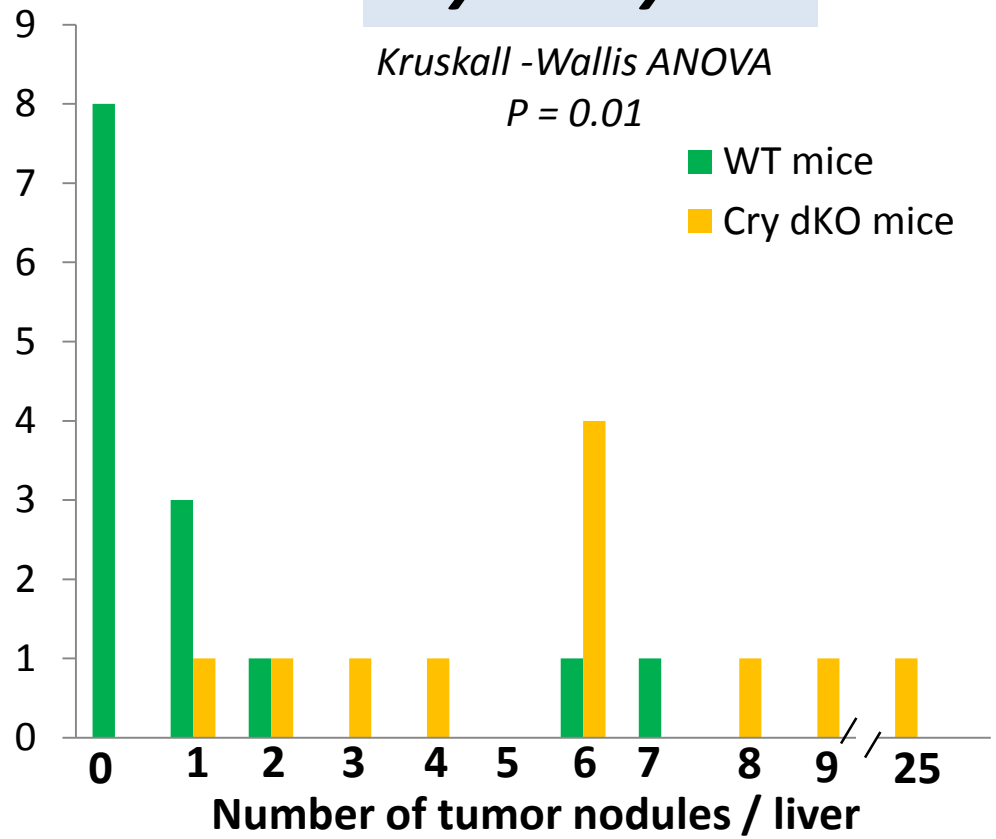


N of mice with >2 nodules

***Wt* : 2/14 (14 %) ; *Cry dKO* : 9/11 (82 %)**

Exact Fisher, p= 0.01

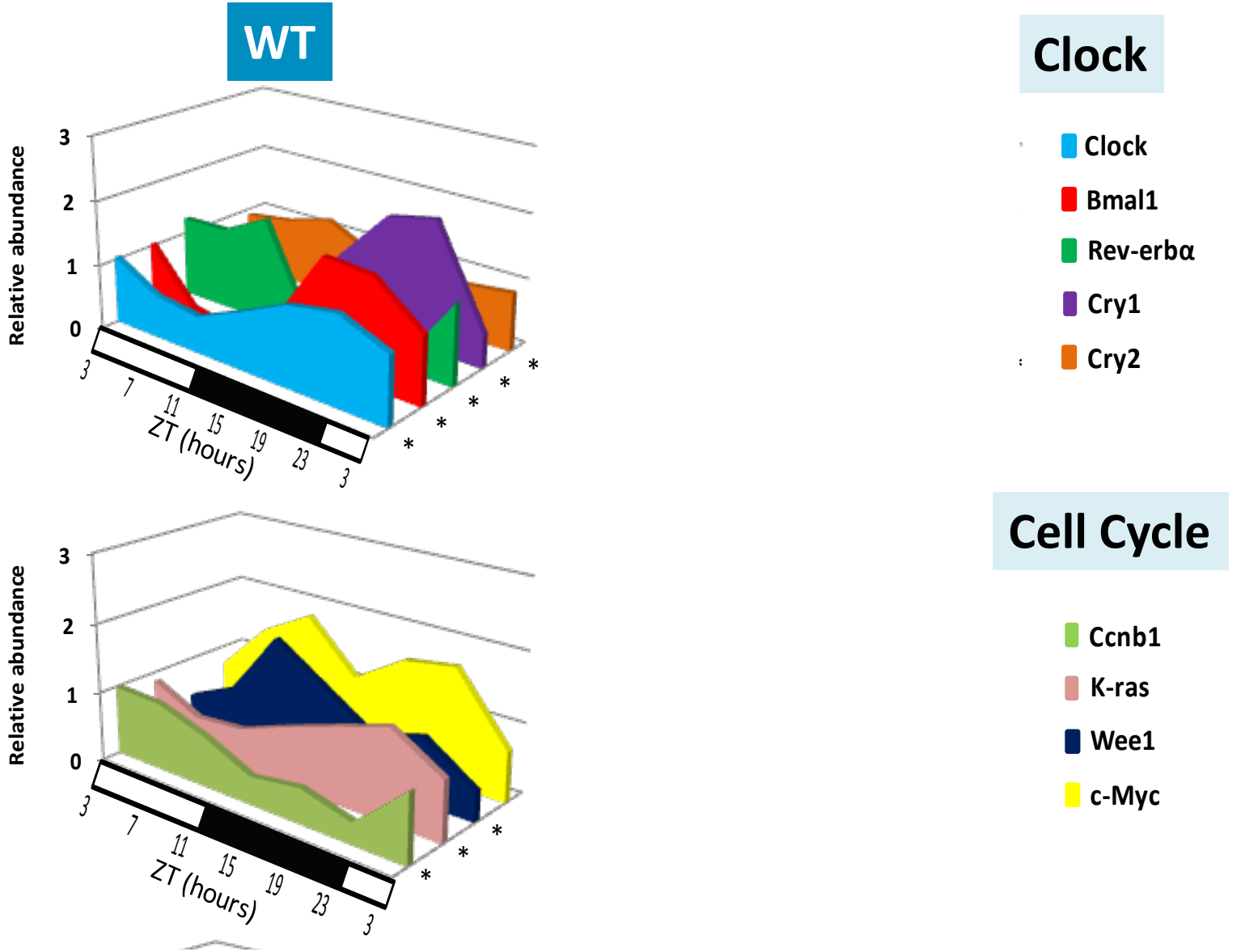
Mteyrek et al. submitted

Early response of circadian gene expressions to DEN

Cancer Initiation

24 h after daily DEN at ZT11 for 3 wks

** 24-h rhythm (p<0.05)*



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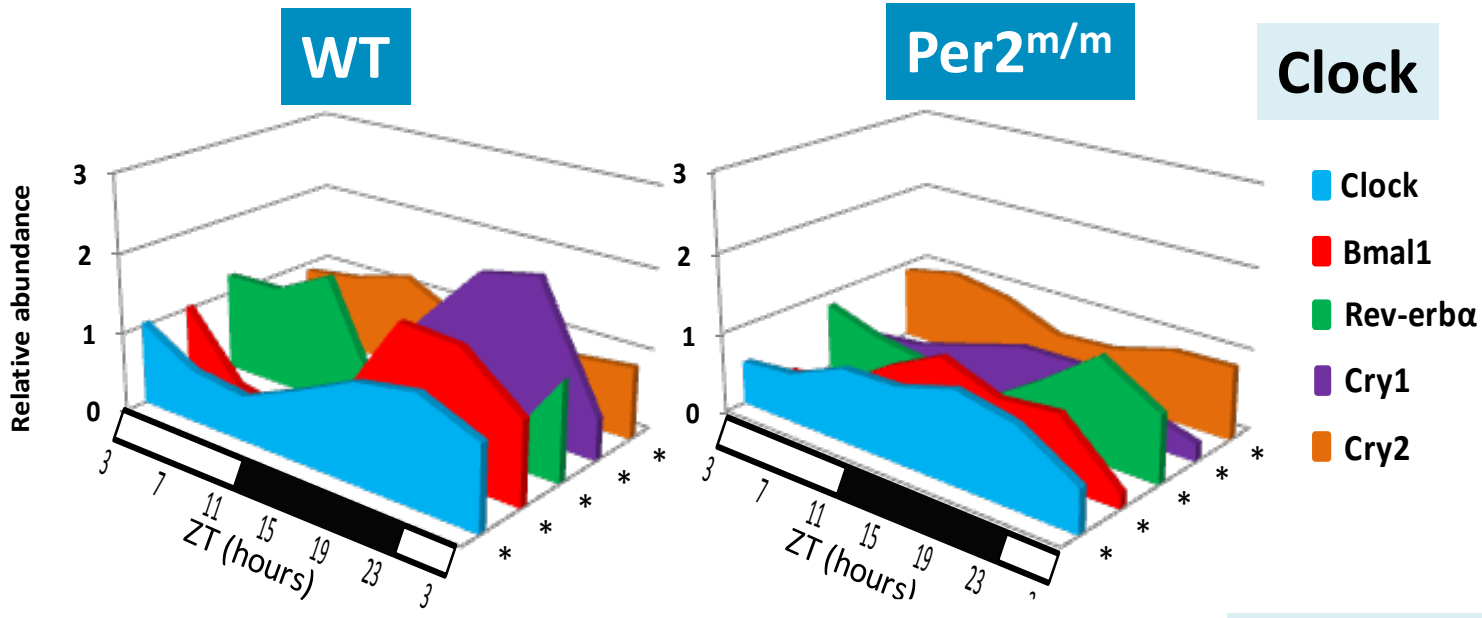

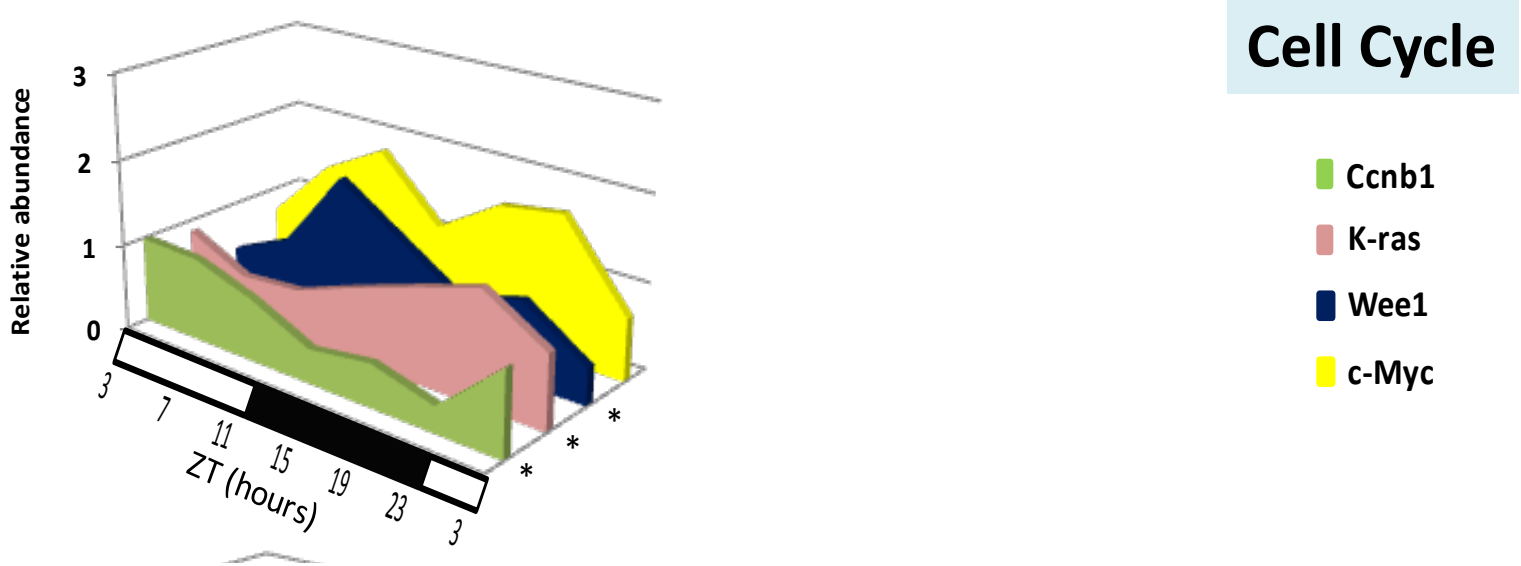

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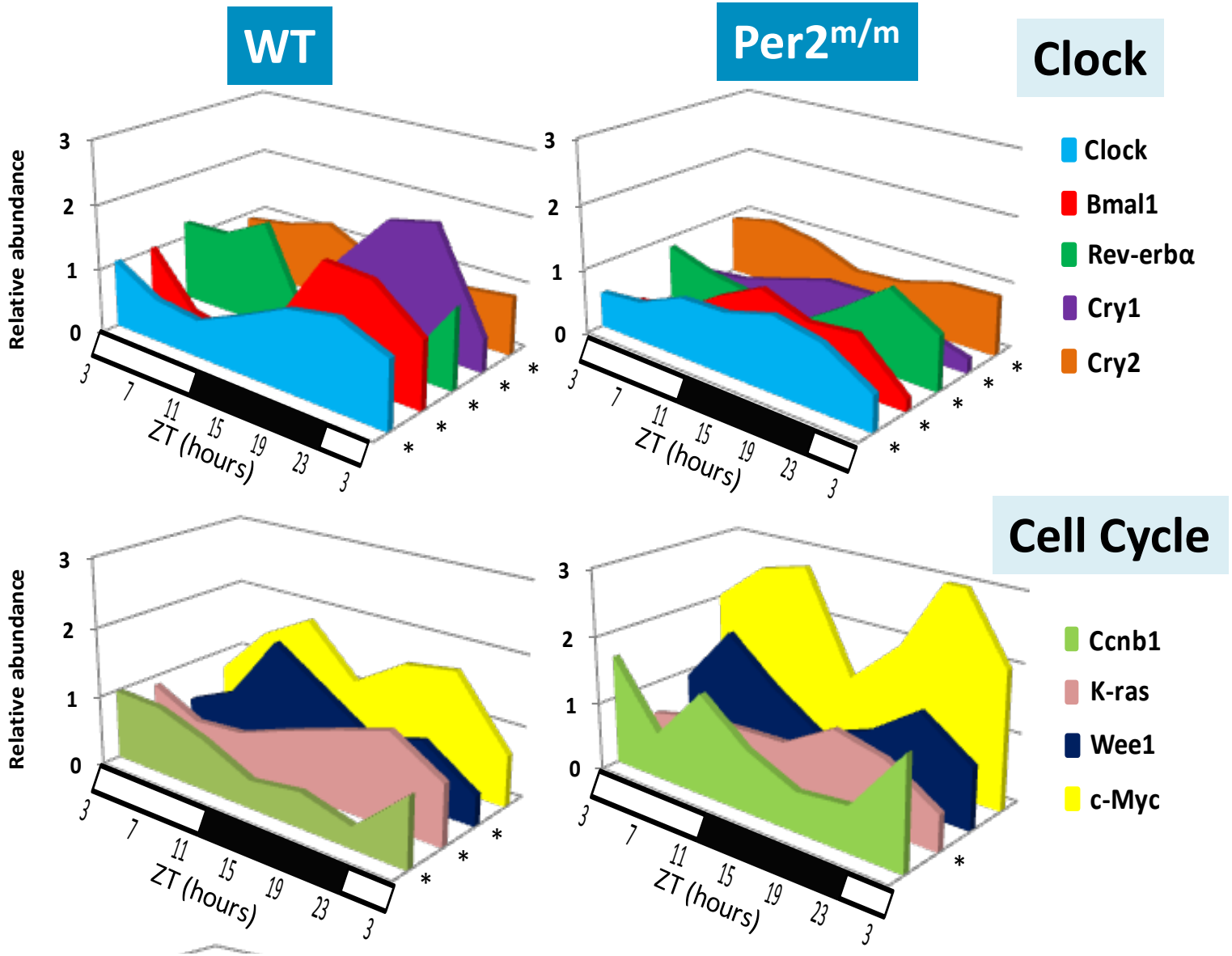

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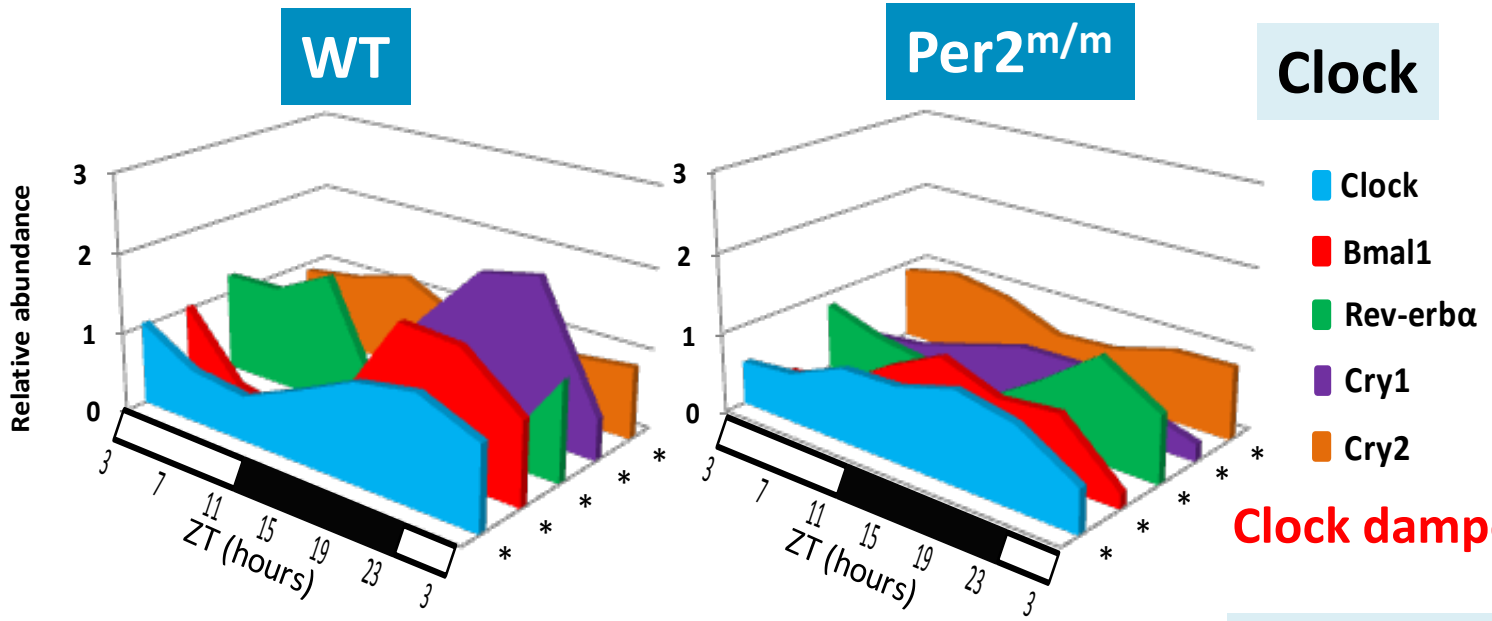
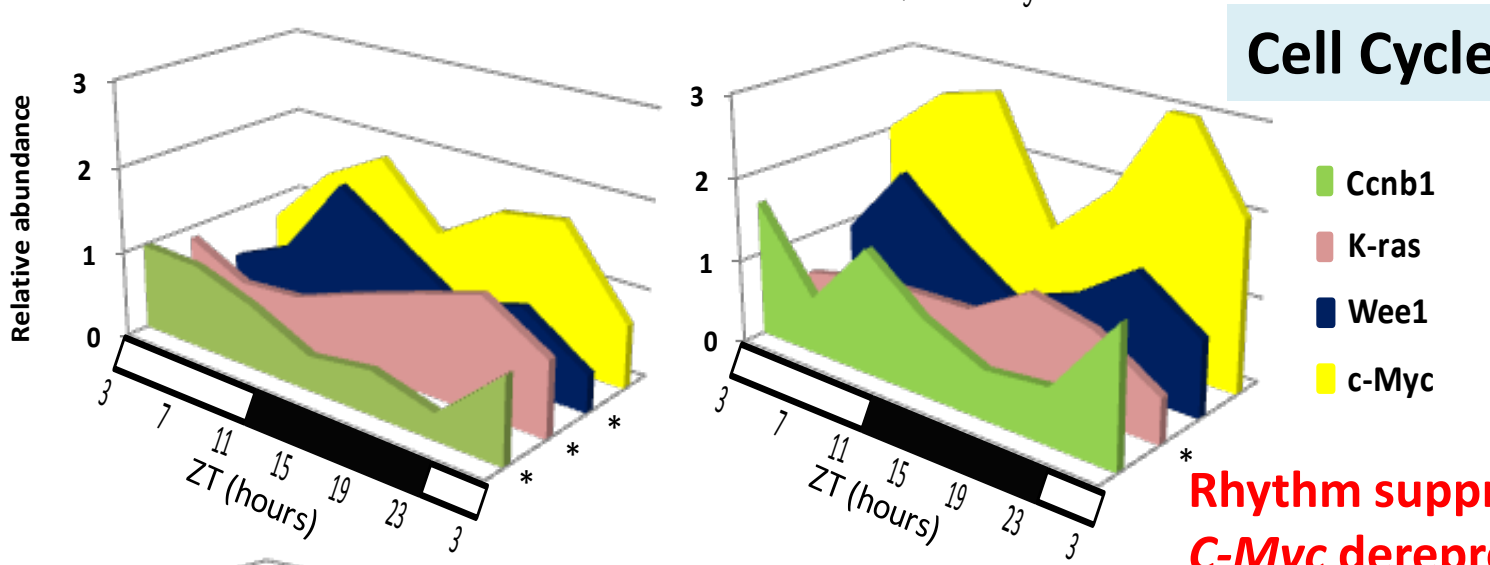


Clock dampening



Rhythm suppression

***C-Myc* derepression**

Mteyrek et al. Submitted

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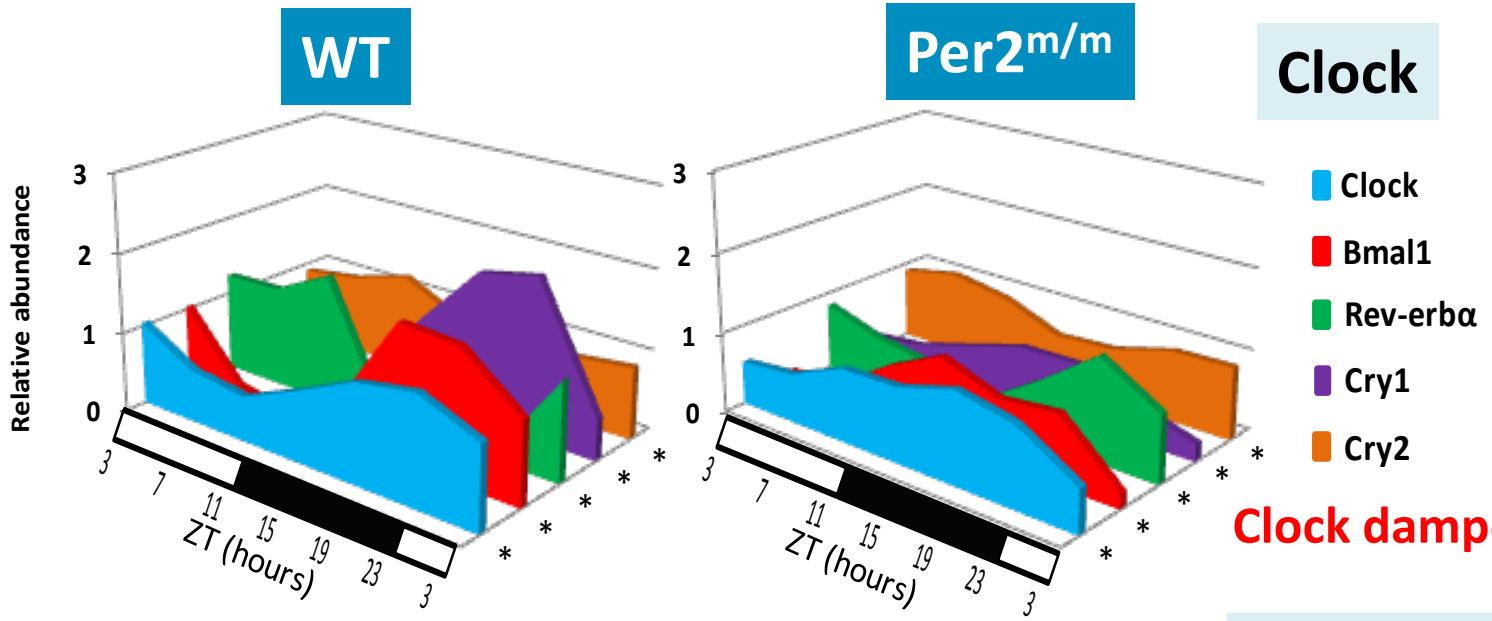

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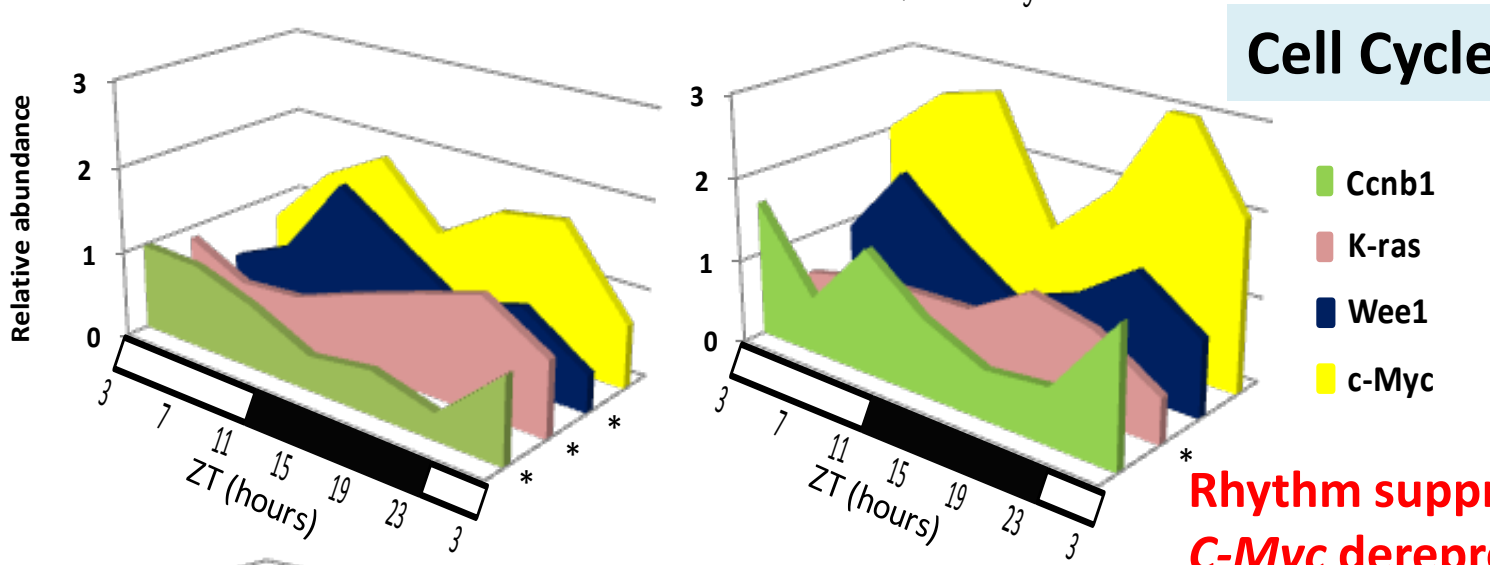

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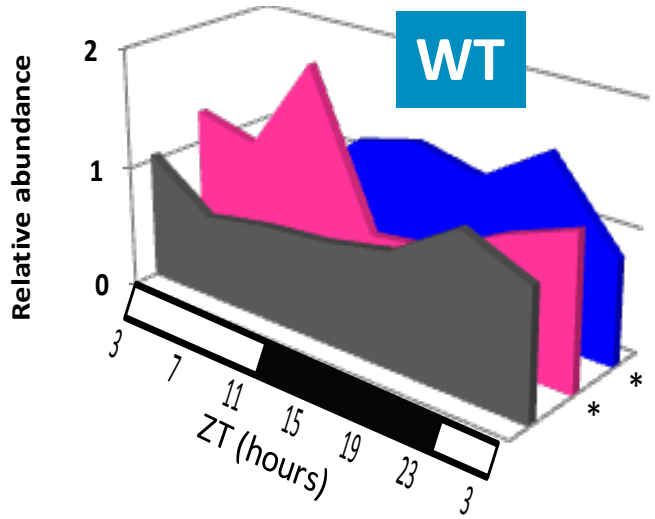
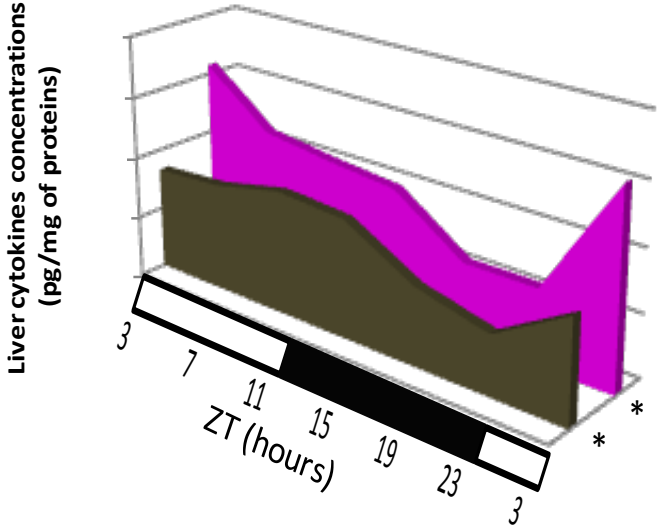


Genomic Instability

- Bcl2
- P53
- ATM



Inflammation

- Il-6
- TNF-α

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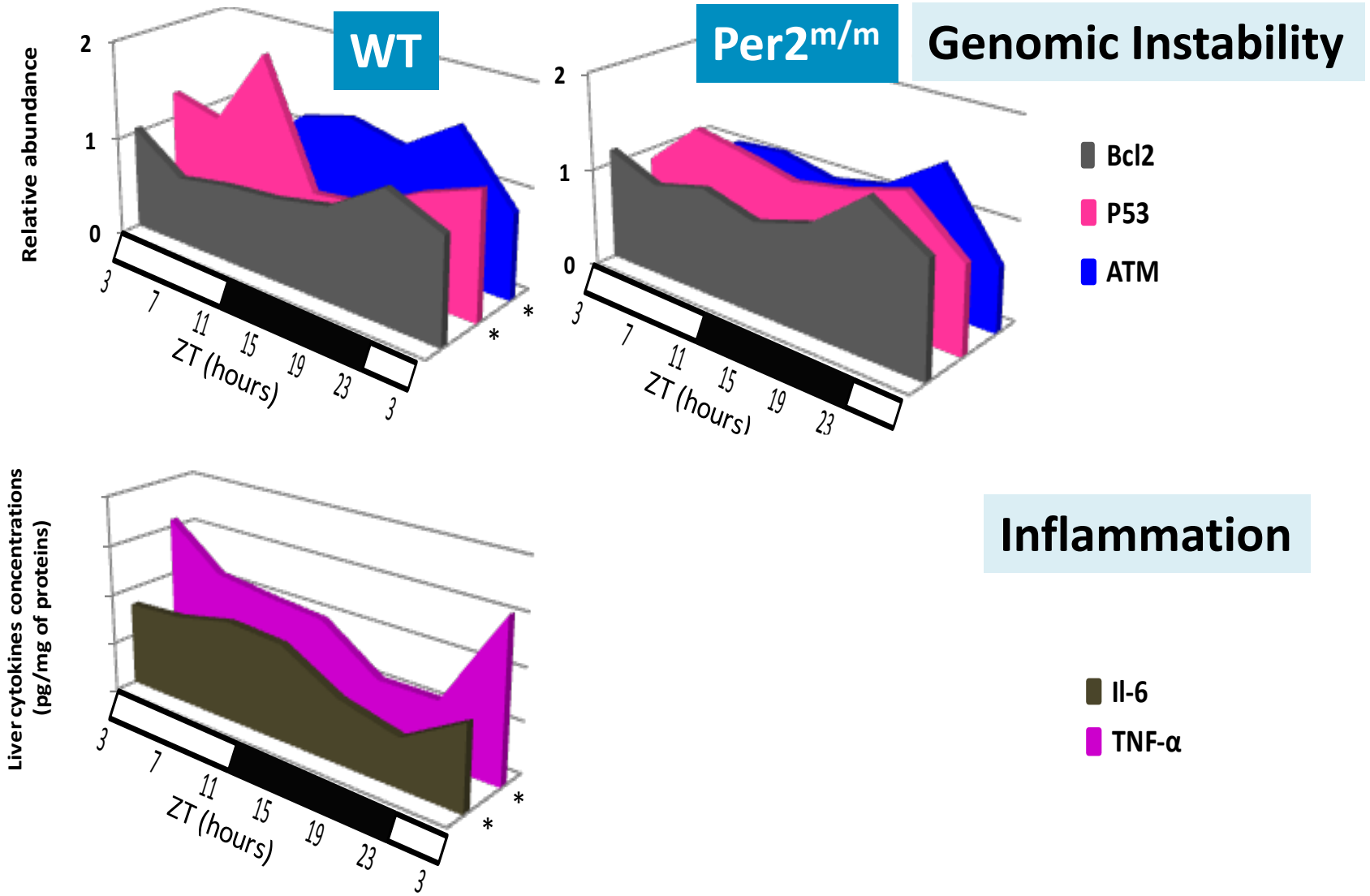

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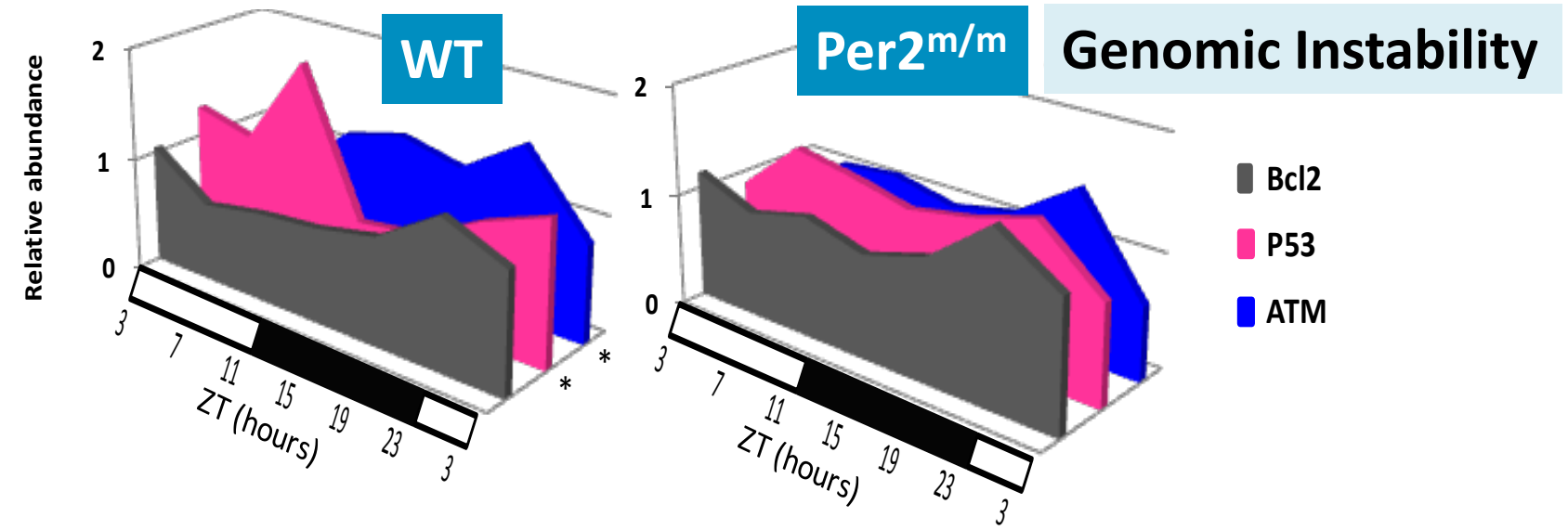
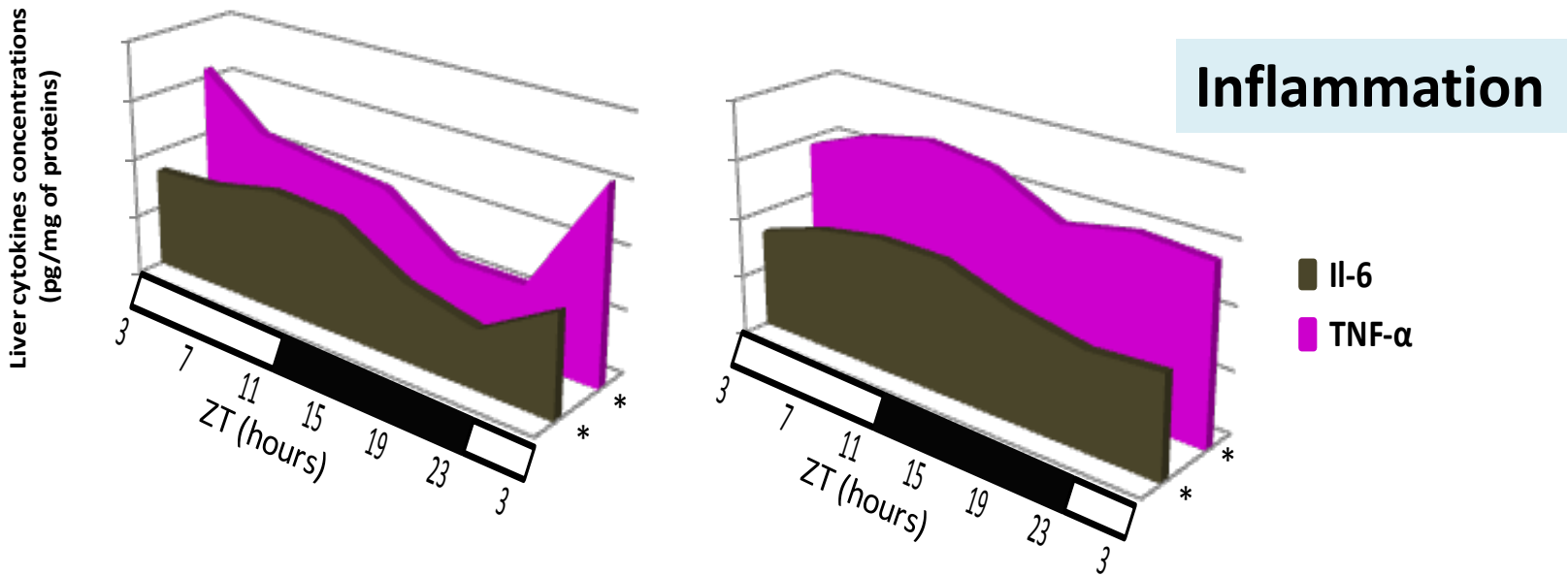


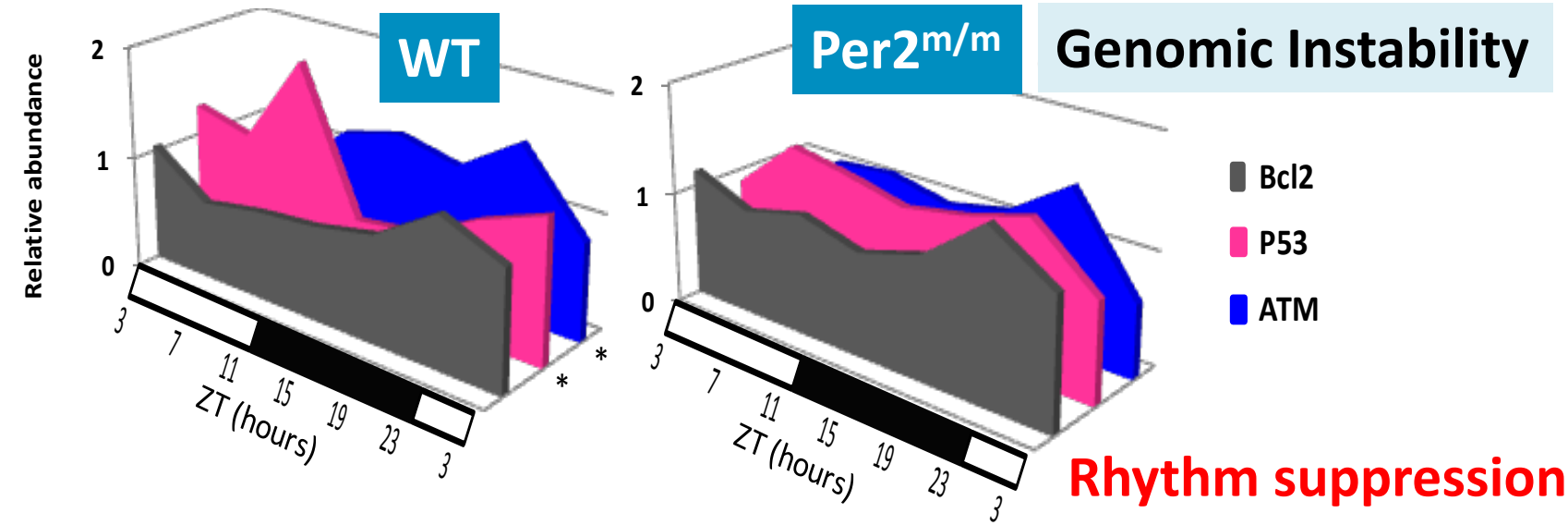


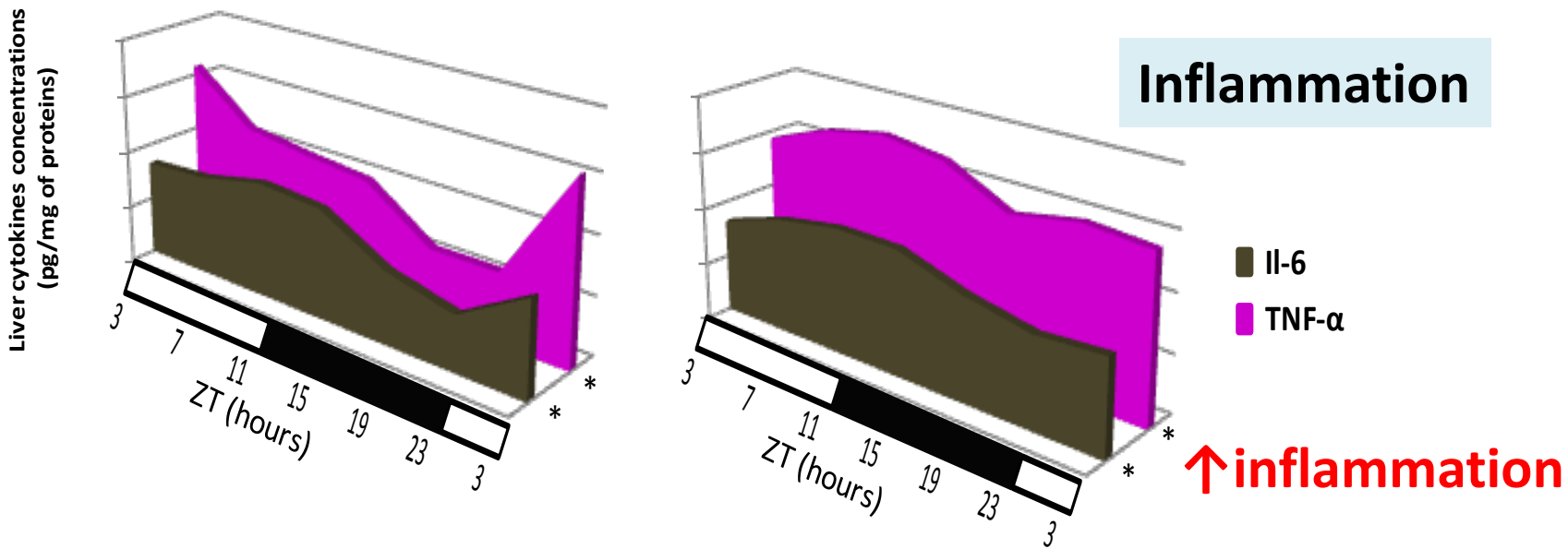
Early response of circadian gene or protein expressions to DEN

Cancer Initiation

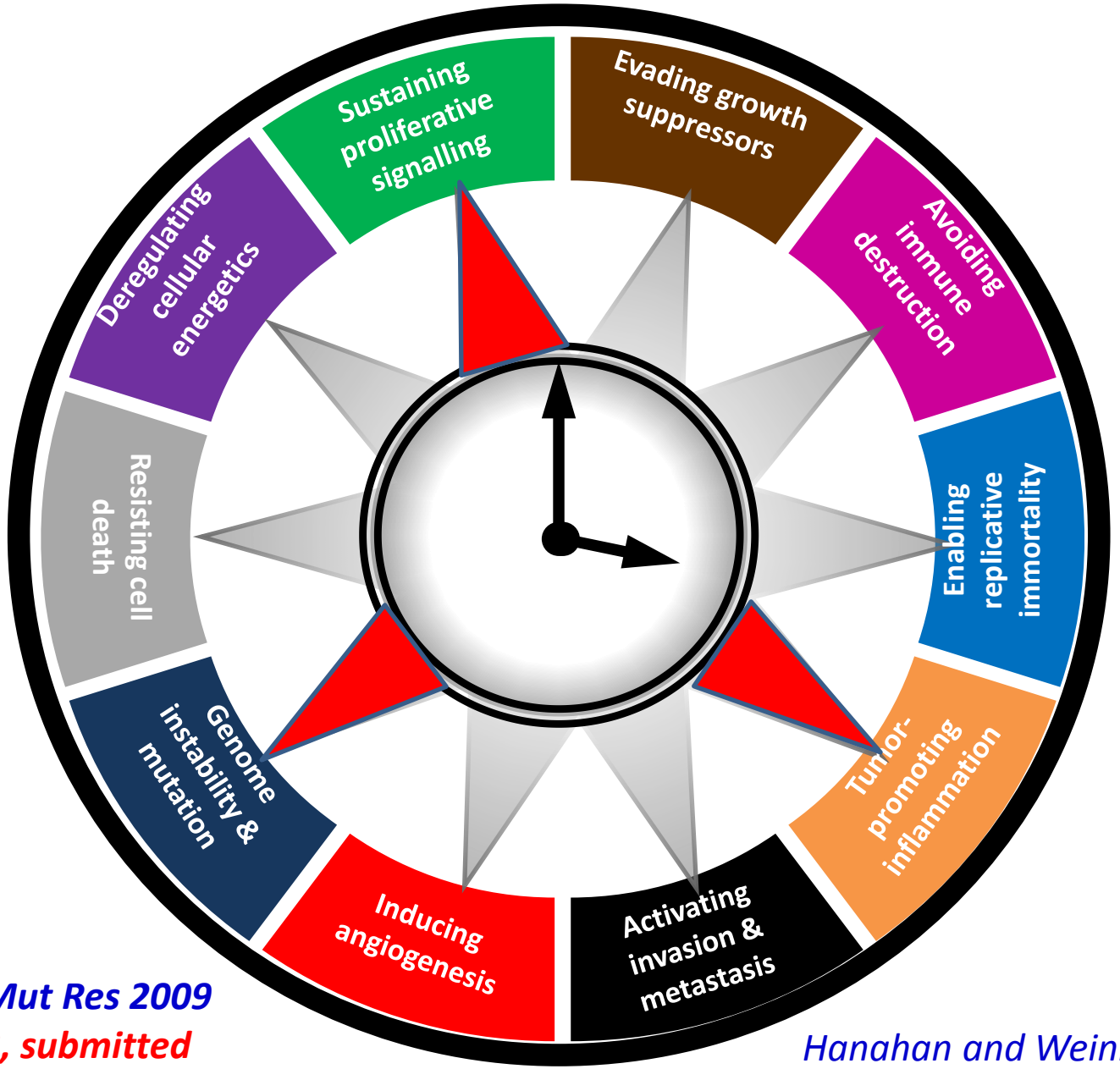
24 h after daily DEN at ZT11 for 3 wks

** 24-h rhythm (p<0.05)*





Circadian Clock On Cancer Hallmarks



Fu et al. Cell 2002
Filipski et al. JNCI 2005, Mut Res 2009
Mteyrek et al. EBRS 2013, submitted

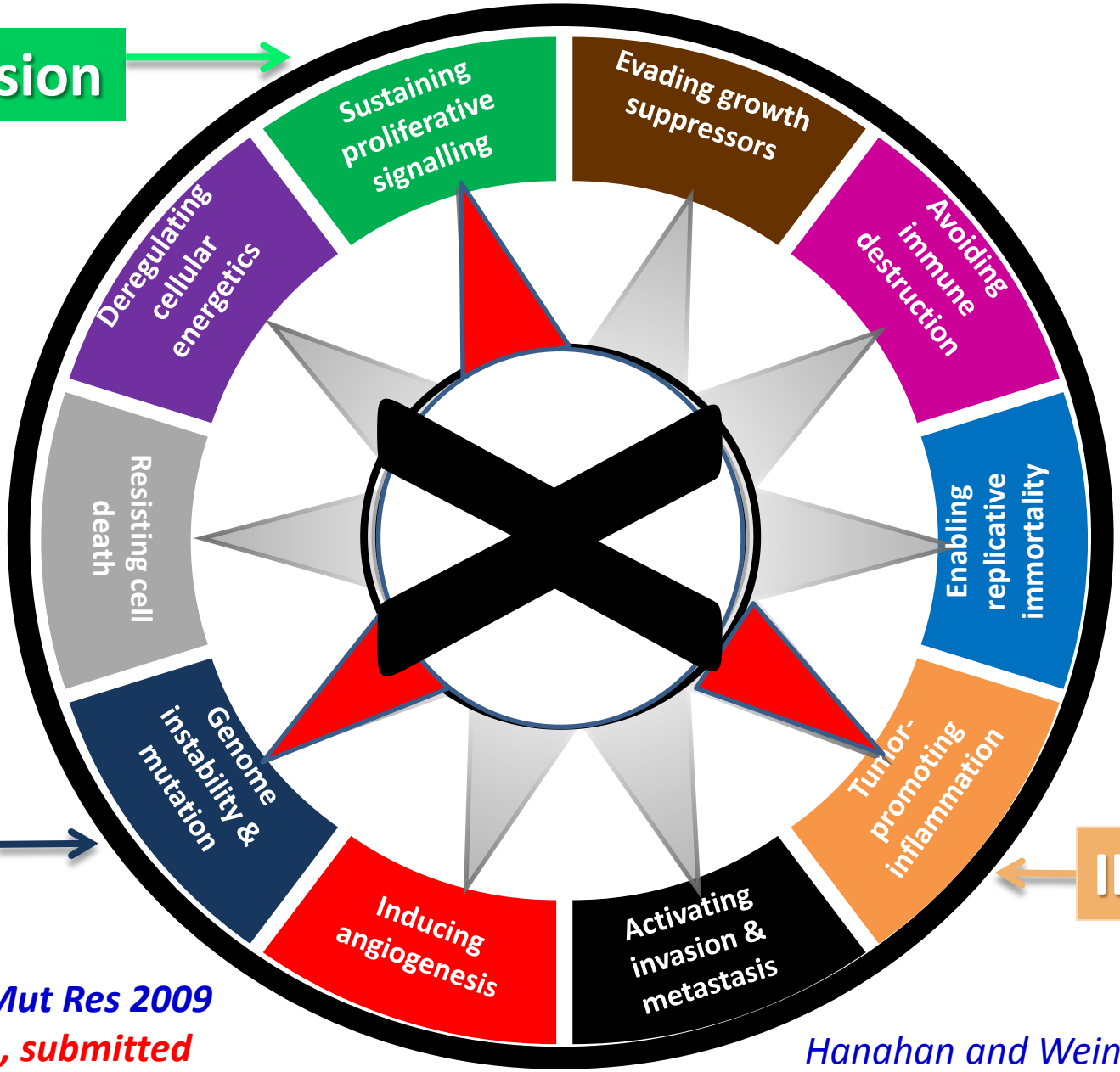
After Hanahan and Weinberg, Cell 2011

Systems Cancer Chronotherapeutics

c-Myc derepression

Circadian Clock On Cancer Hallmarks

P53 repression



IL6, TNFα

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Filipski et al. JNCI 2005, Mut Res 2009
Mteyrek et al. EBRS 2013, submitted

After Hanahan and Weinberg, Cell 2011

Host circadian disruption speeds up cancer

Host circadian reinforcement inhibits cancer progression

Host circadian disruption speeds up cancer

Host circadian reinforcement inhibits cancer progression

Host circadian disruption speeds up cancer

Host circadian reinforcement inhibits cancer progression

Host clocks control cancer

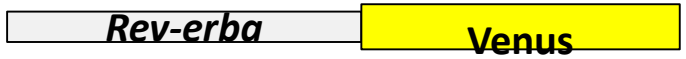
In vitro cancer chronopharmacology

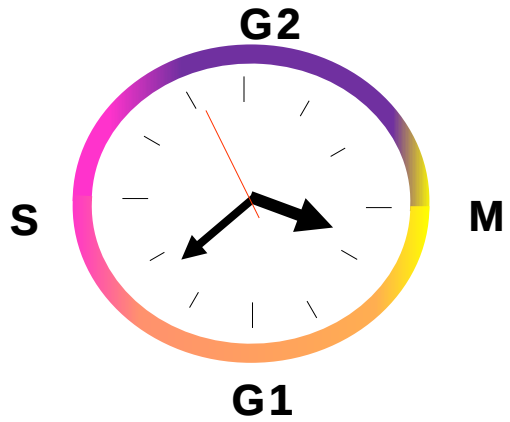
- **Coupling of circadian clock and cell cycle at single cell level**
- ***In vitro* chronopharmacology of anticancer drug irinotecan**

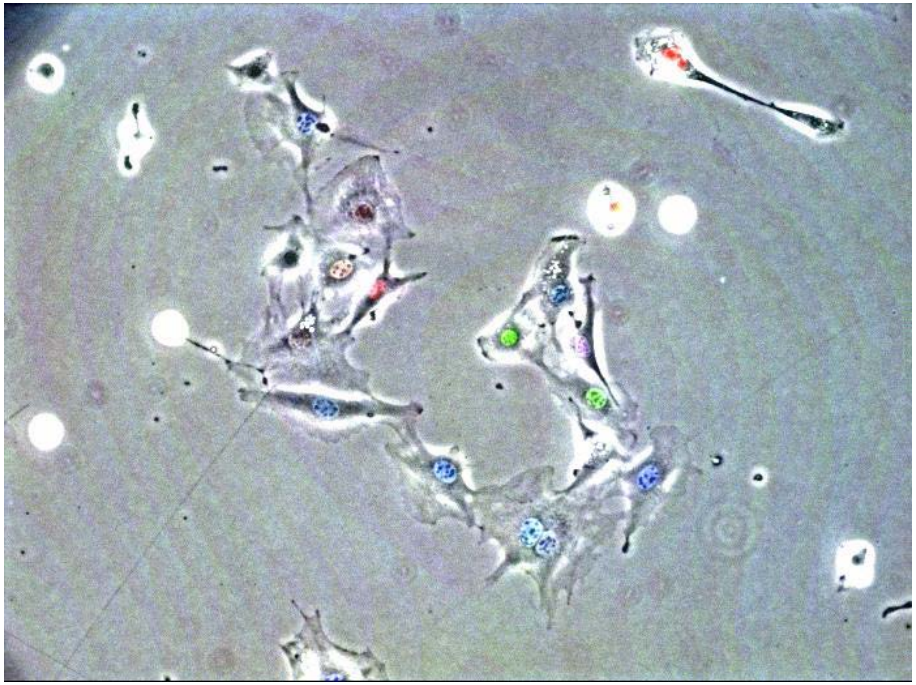
Modified FUCCI reporter system introduced into NIH3T3 cells carrying the Rev-erbα-Venus reporter

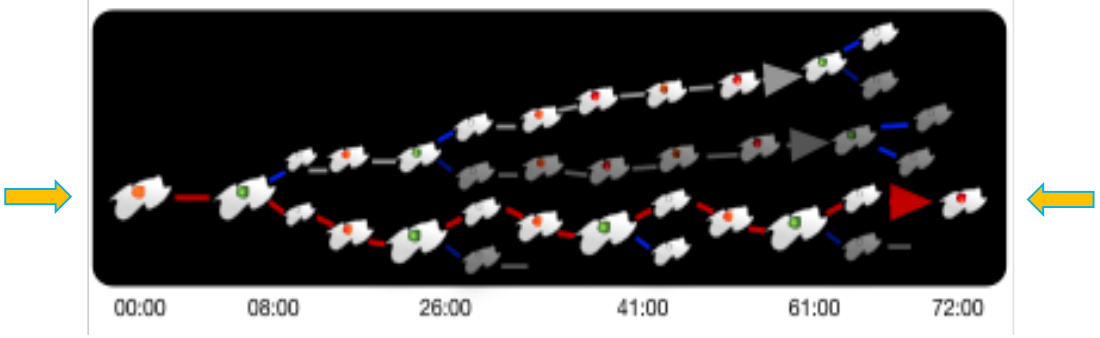
Cell Cycle probes

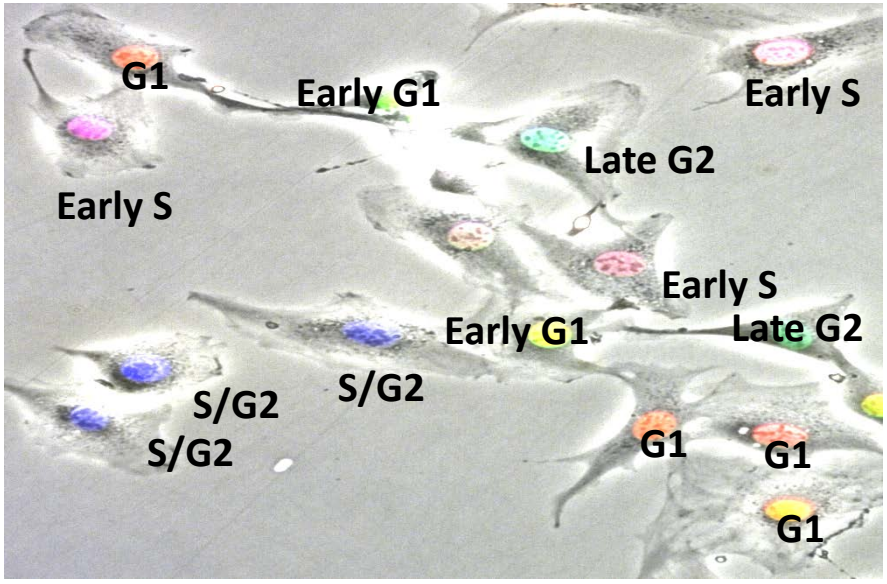
Clock probes





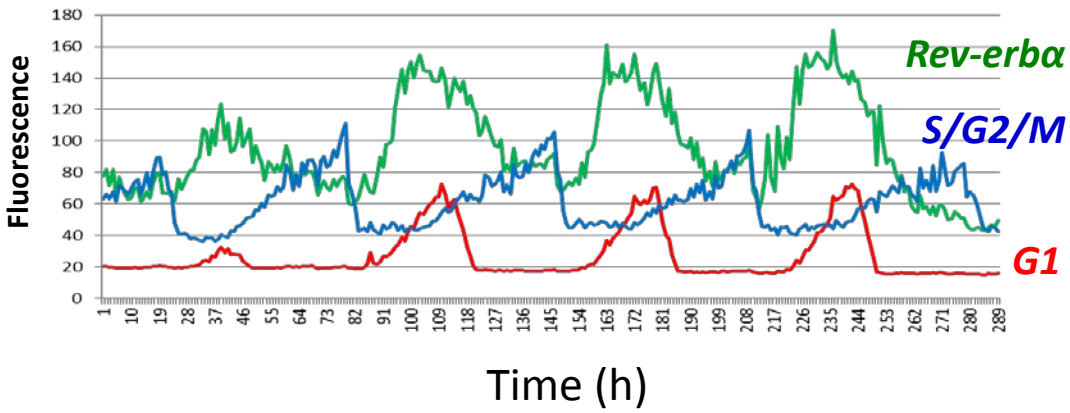


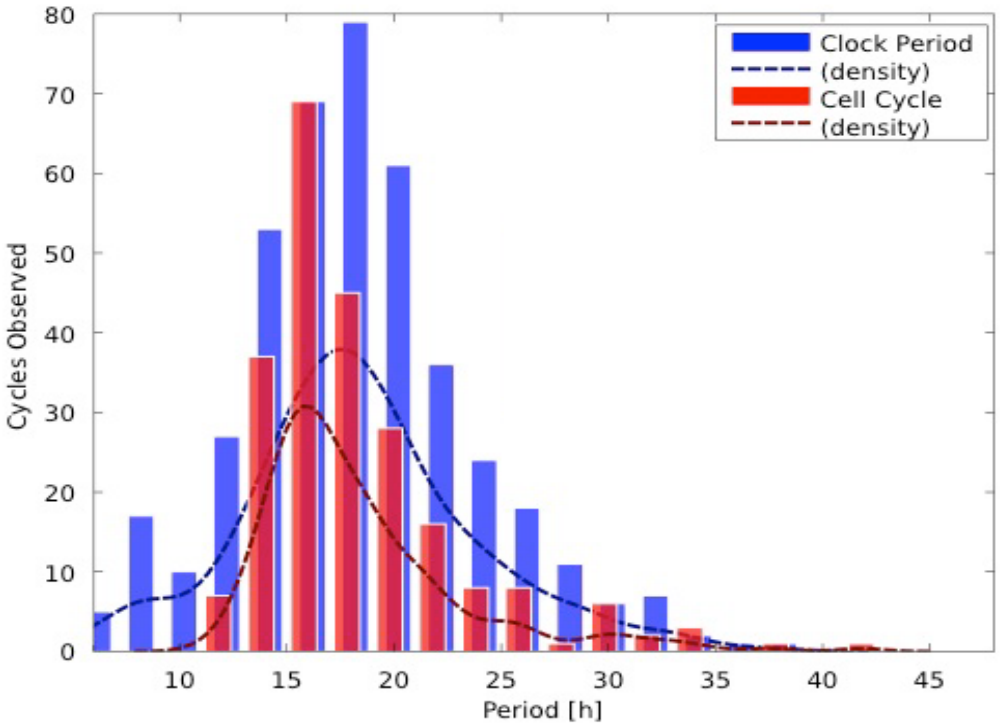




Feillet et al. PNAS (in press)

1:1 phase-locked Circadian Clock and Cell Cycle in undisturbed NIH3T3 cells





Circadian Clock period	18.6 h ± 0.6
Cell Cycle period	18.2 h ± 0.5

15% FBS in culture medium

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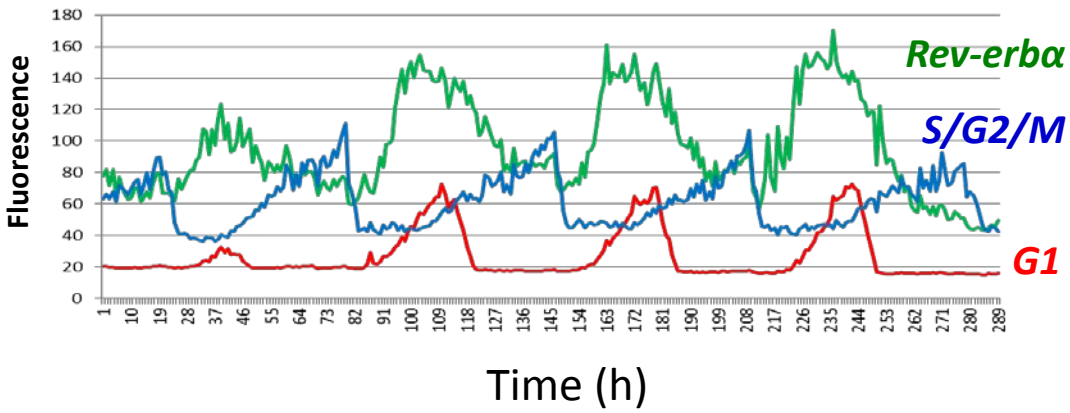
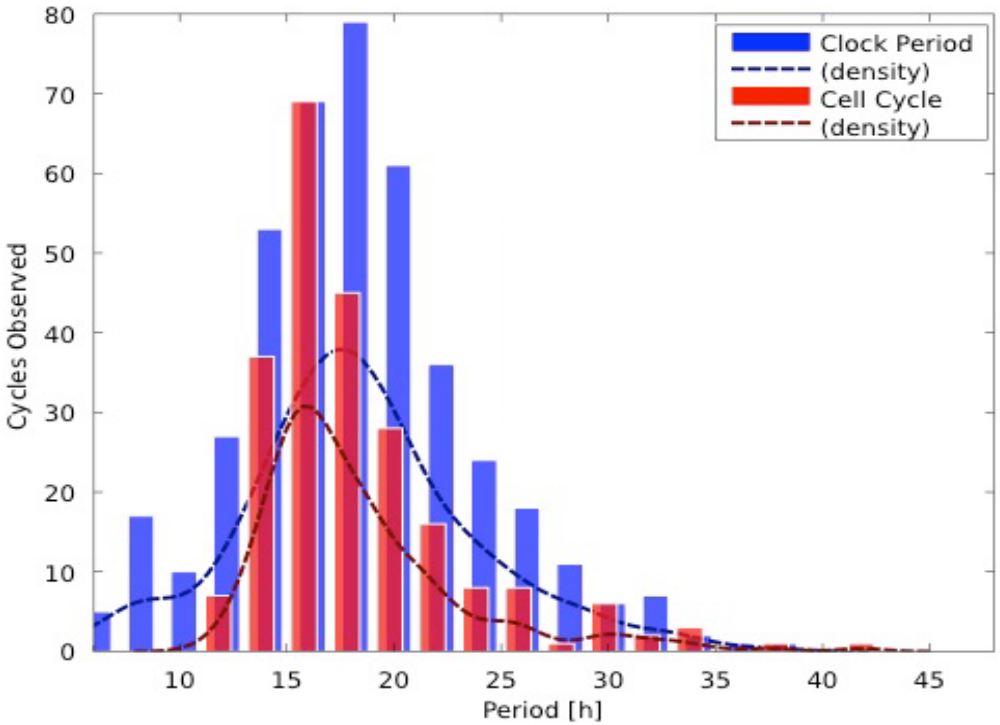


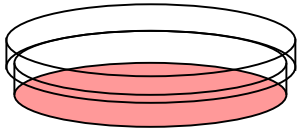


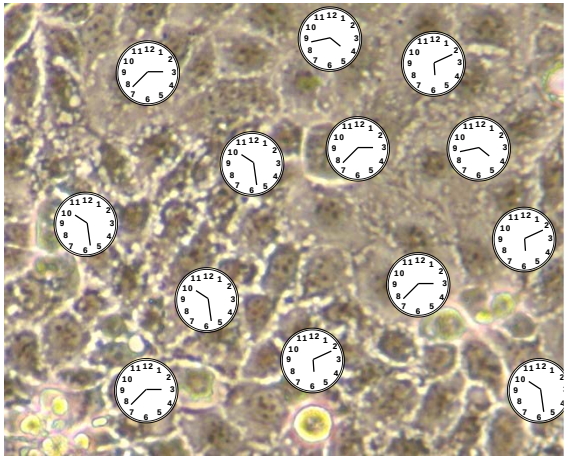
Peak transcriptional activity of clock gene *Rev-erb α* predicts timing of cell division

15% FBS in culture medium

Feillet et al. PNAS (in press)

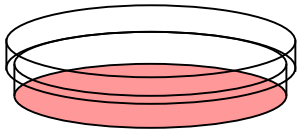
Cell culture





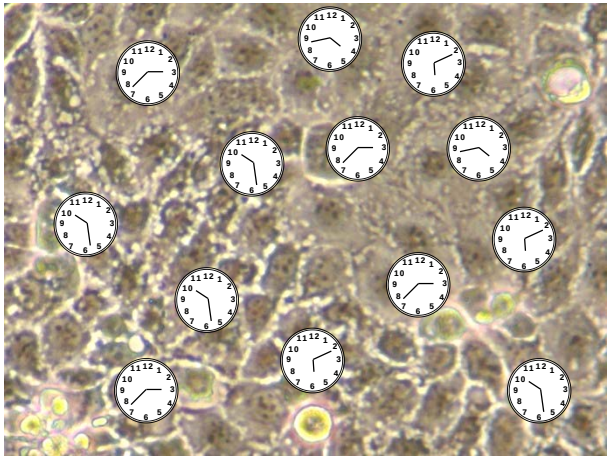
Systems Cancer Chronotherapeutics

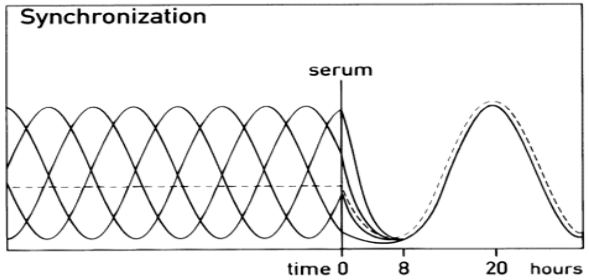
Cell culture **2 h** **Circadian synchronisation**

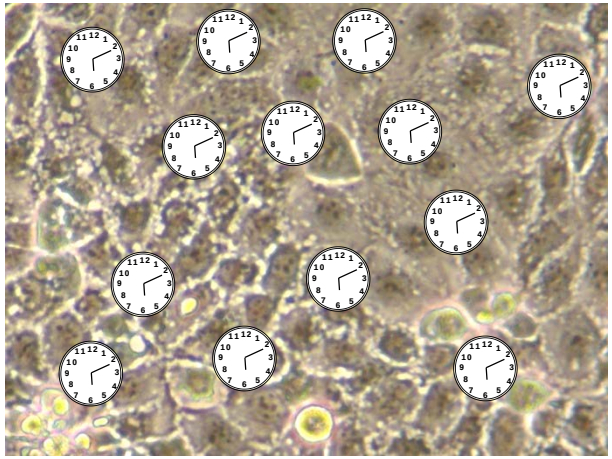


- 50% FCS or
- Dexamethasone

Samples every 10 min- 4 h for 48-72 h

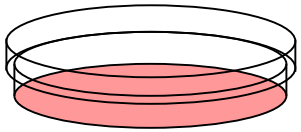






Systems Cancer Chronotherapeutics

Cell culture → **2 h** → **Circadian synchronisation**



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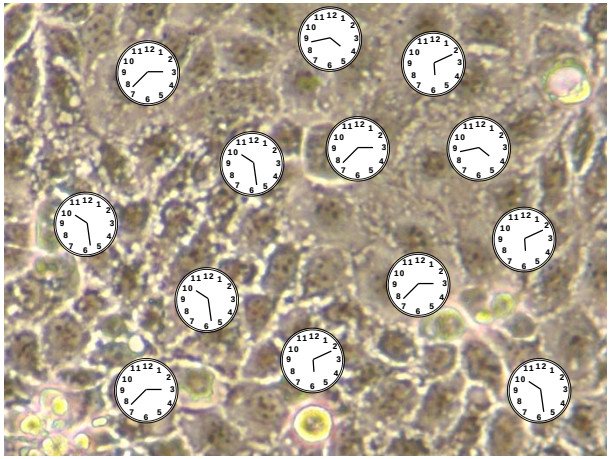
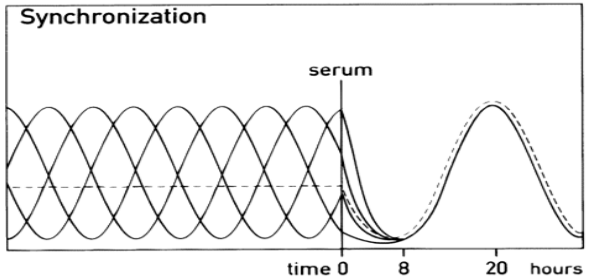
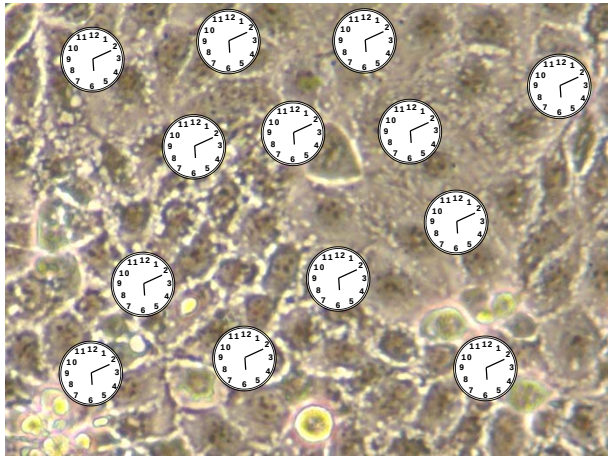




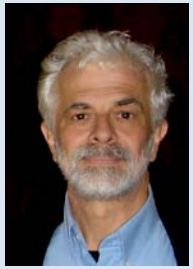


S Dulong

V Roche

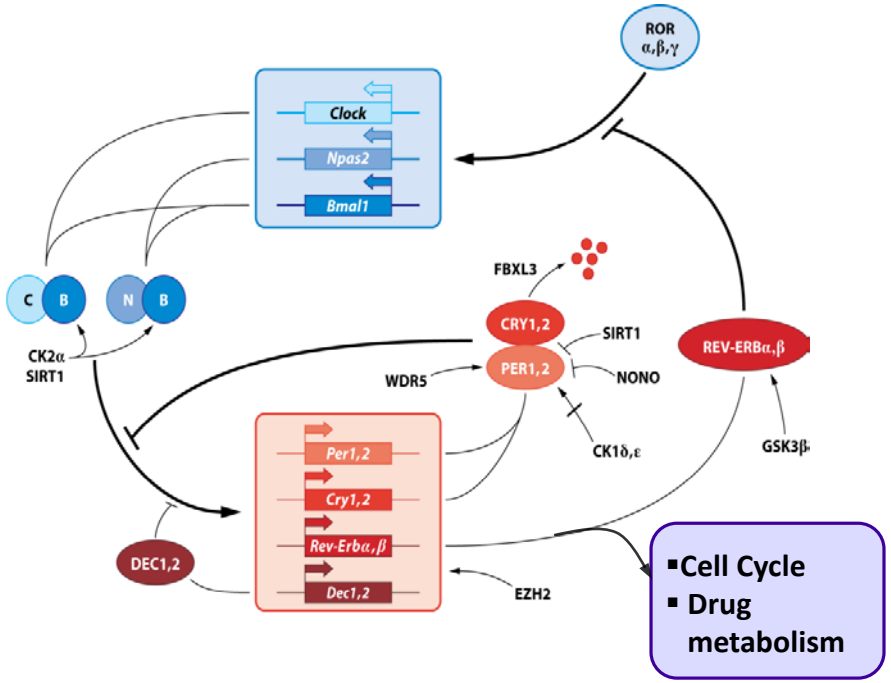
N Ozturk

A Ballesta


J Clairambault

***In vitro-in silico* chronopharmacology of anticancer drugs**

Genetic clock in human CaCo-2* cell populations



Lévi et al., Annu Rev Pharm Toxicol 2010; 50: 377-421

* Human colorectal cancer cell line: ~ mimicks colon epithelium at confluence

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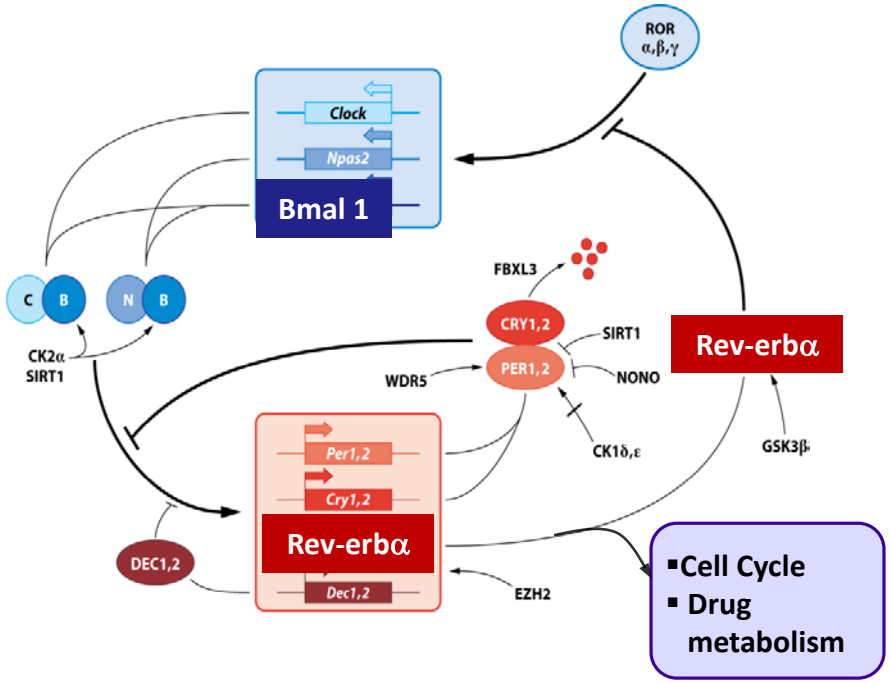

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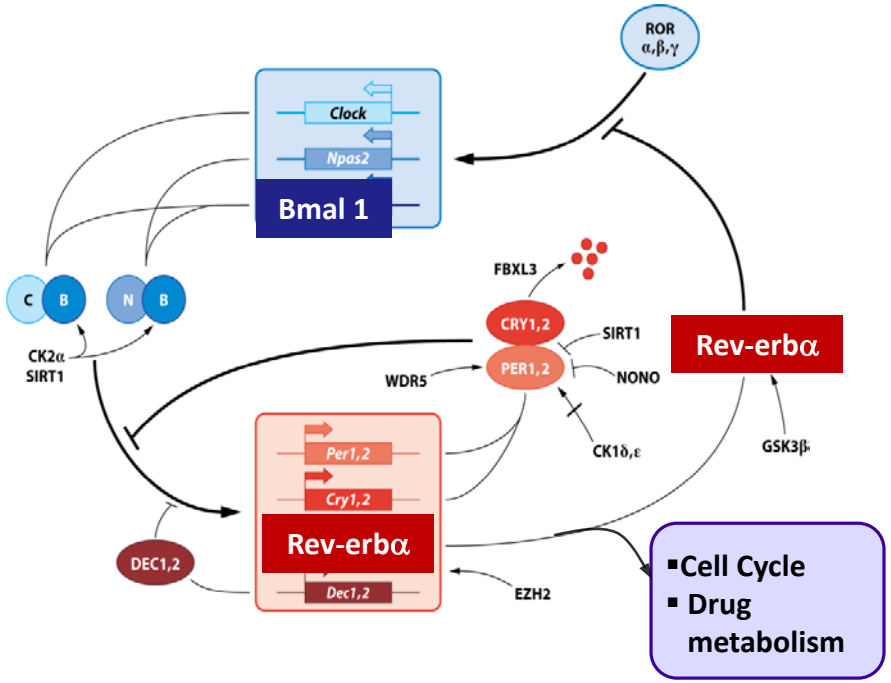

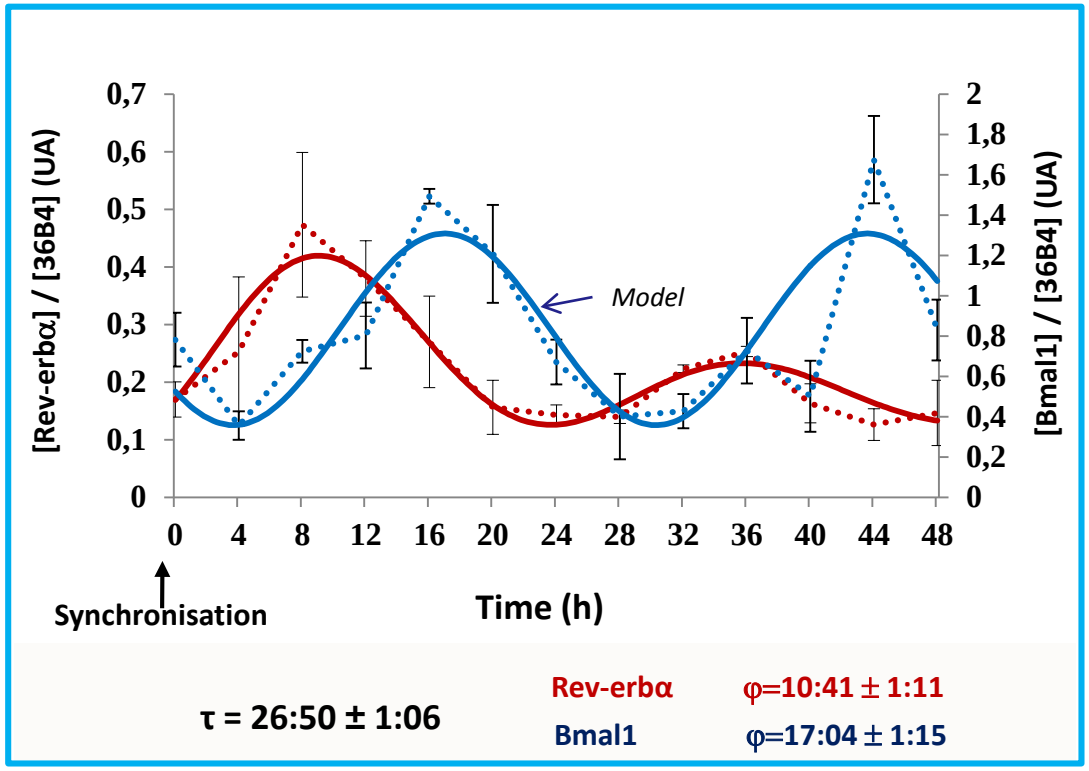

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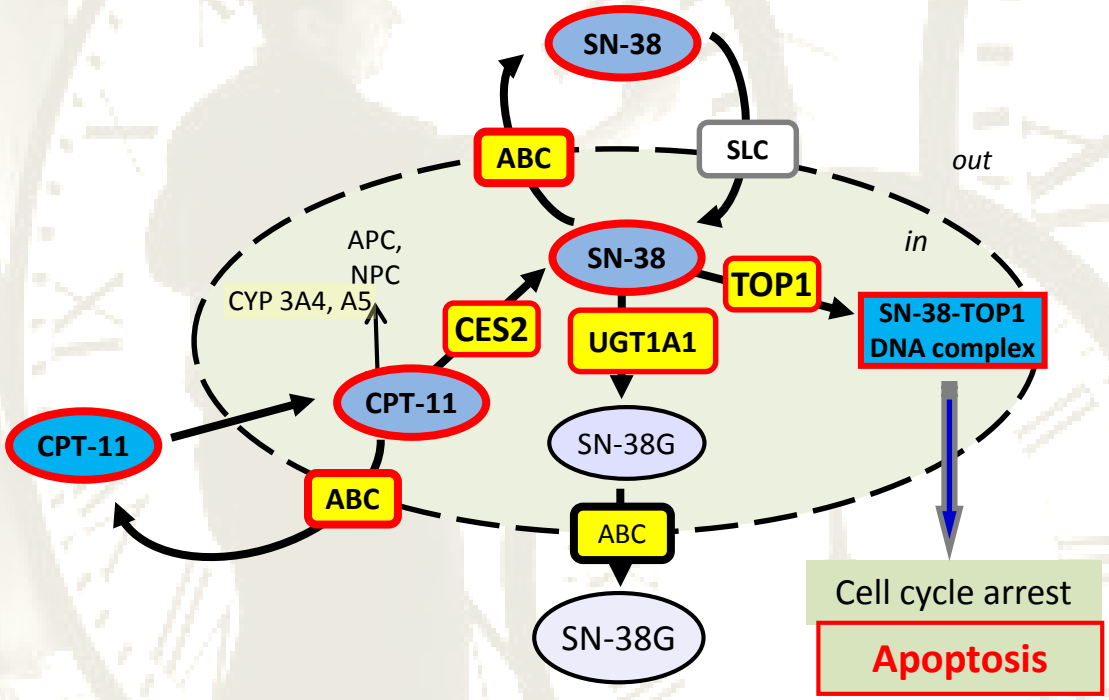
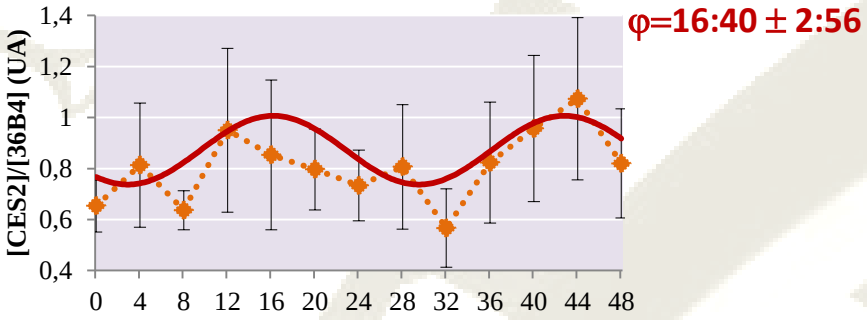
A Combined Experimental and Mathematical Approach for Molecular-based Optimization of Irinotecan Circadian Delivery





Synchronisation

Time (h)

Variables studied in synchronized and non synchronized Caco-2 cells

Ballesta et al., Plos Comput Biol, 2011; e1002143

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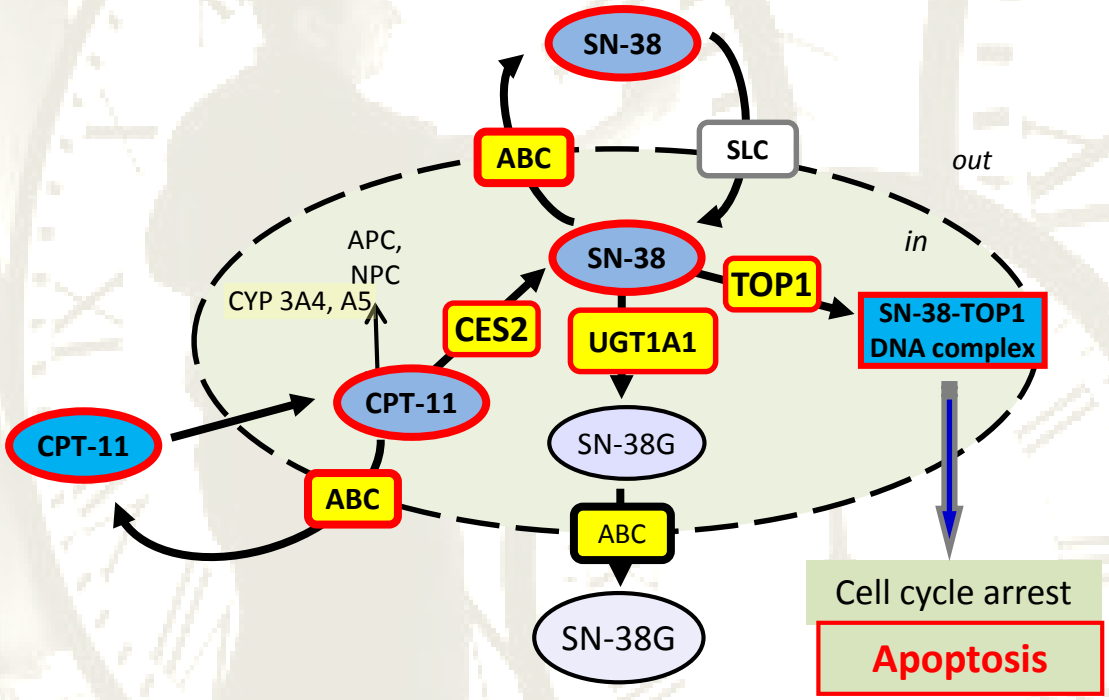

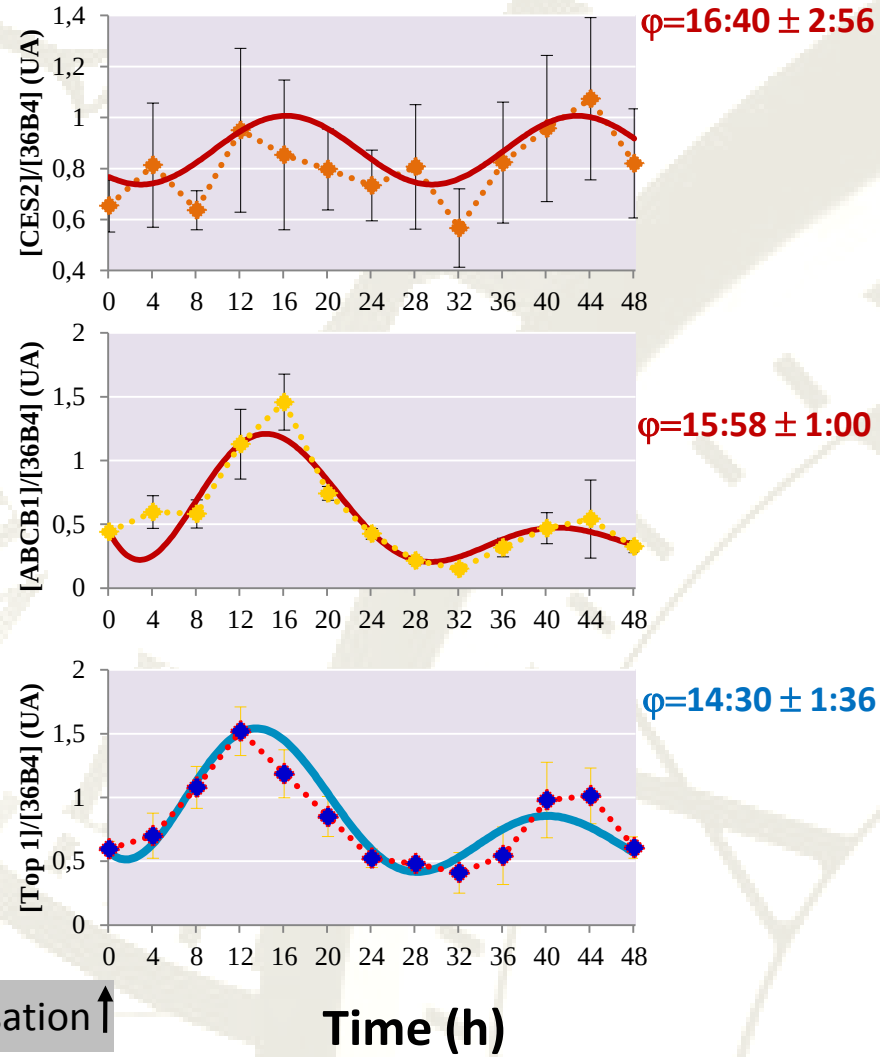

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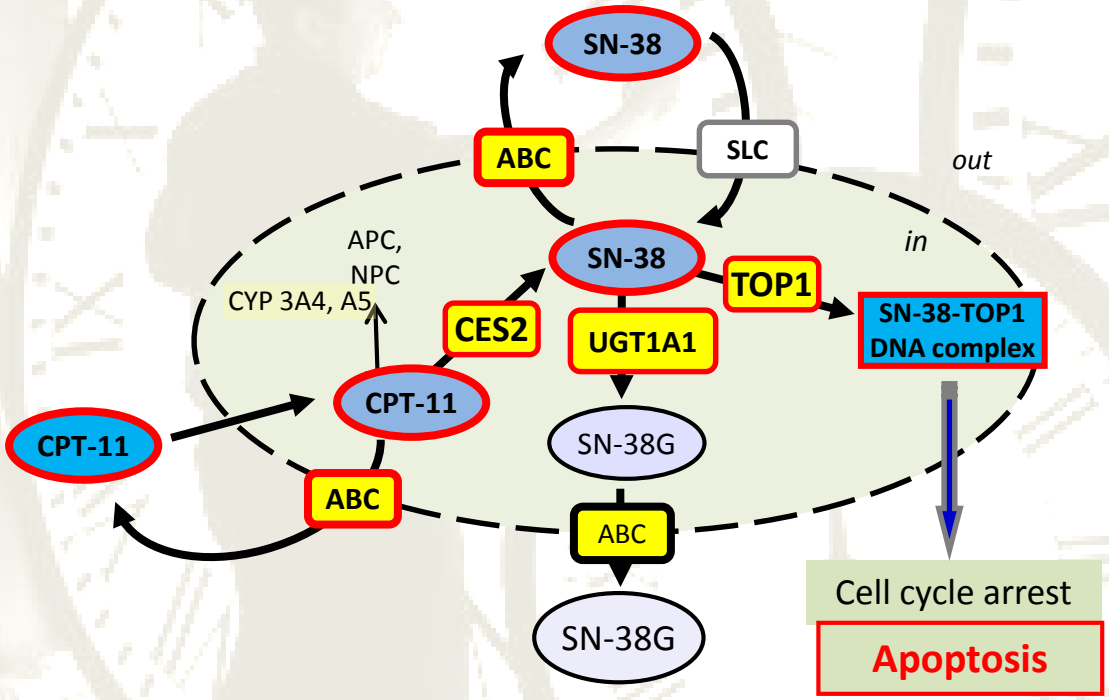

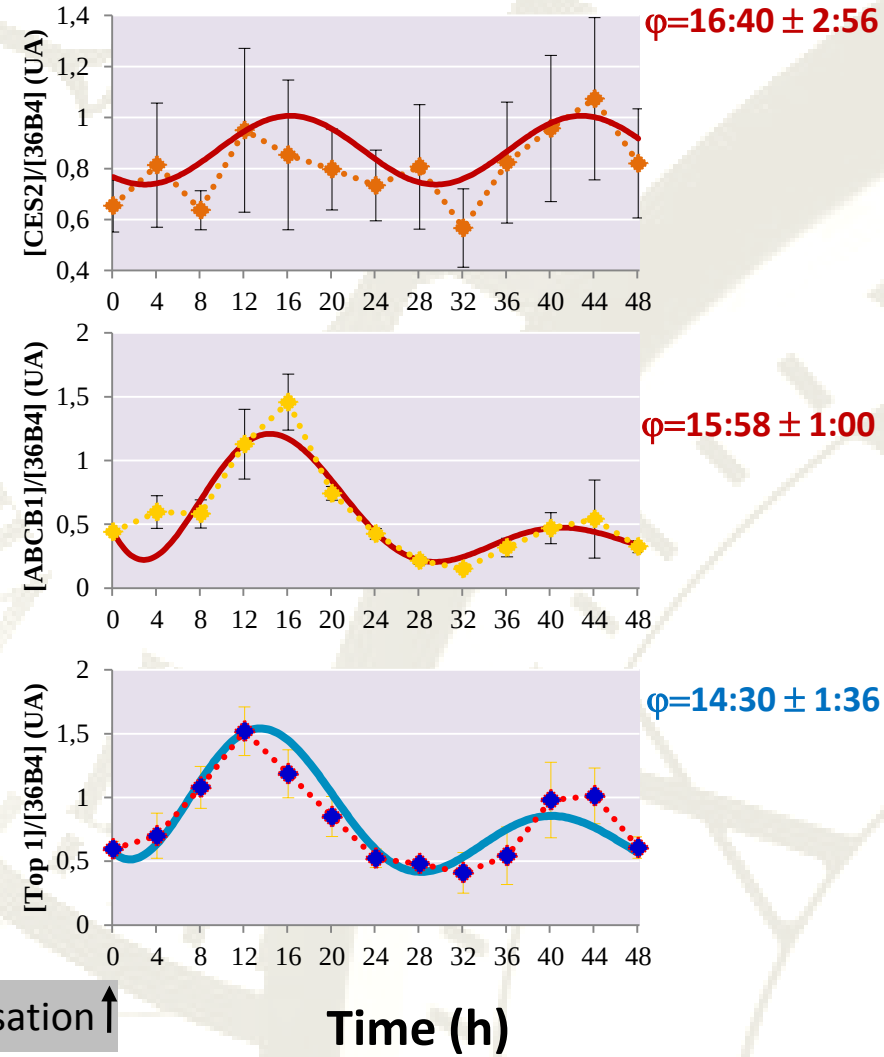

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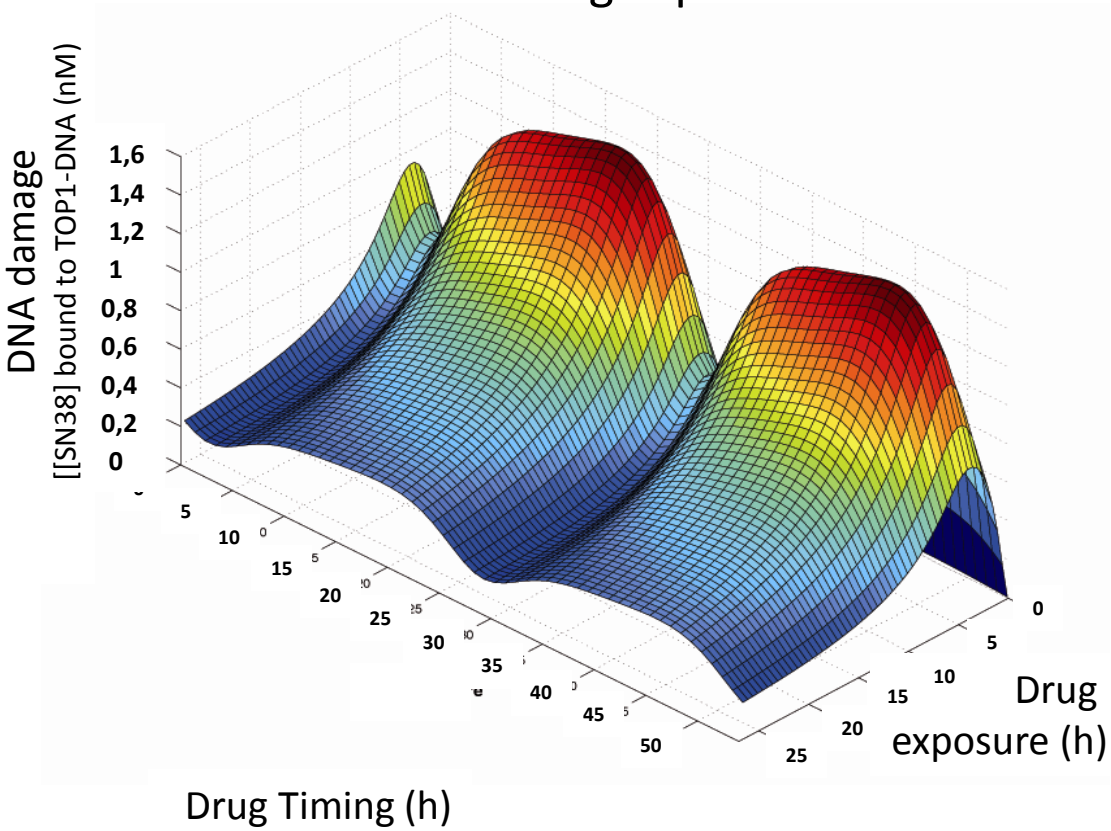
Irinotecan chronotherapeutic model

Molecular PD according to circadian drug timing and drug exposure duration



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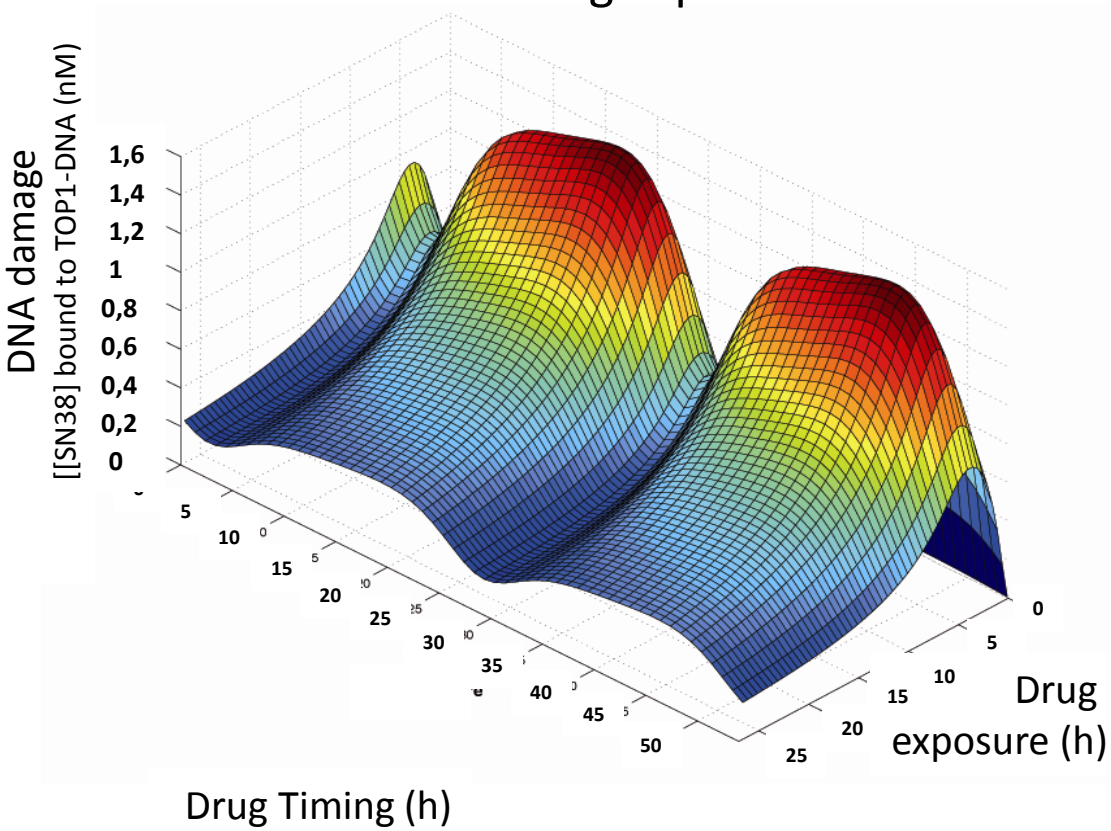

Model validation

- Molecular clock
- Cellular chronoPK
- Cellular chronoPD
- *In vitro* chronotoxicity

Model adjustment

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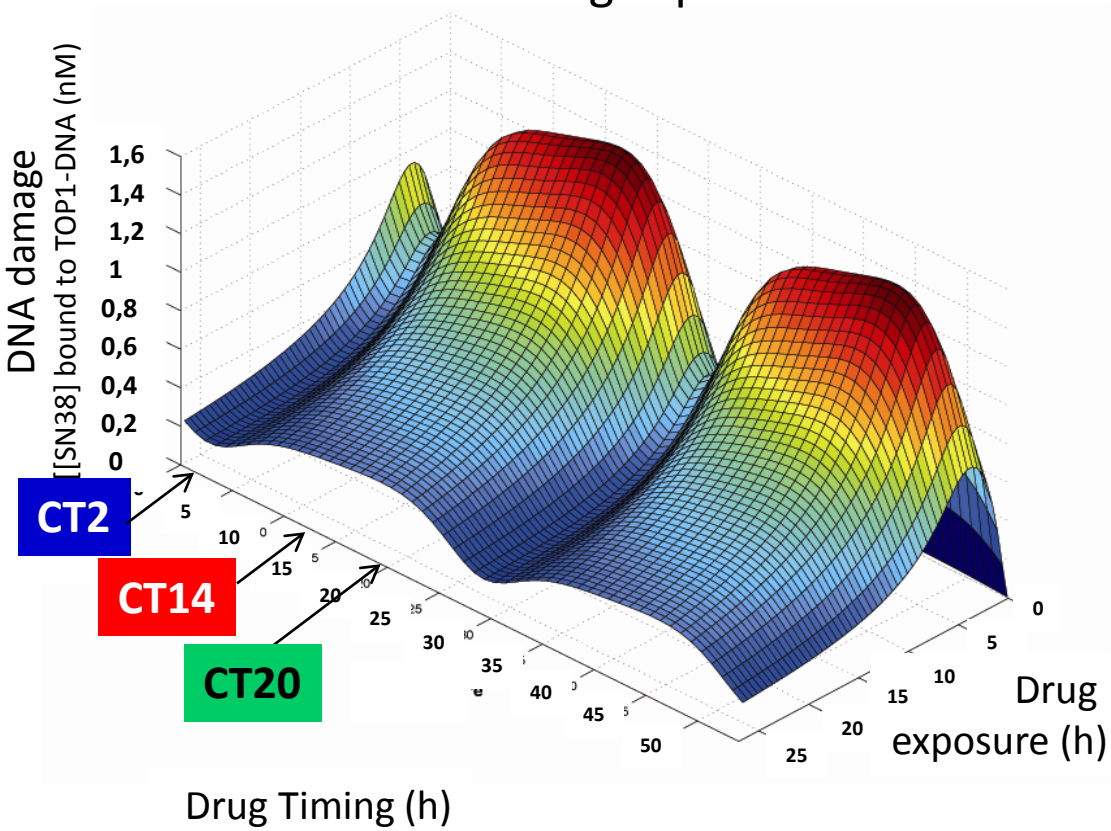

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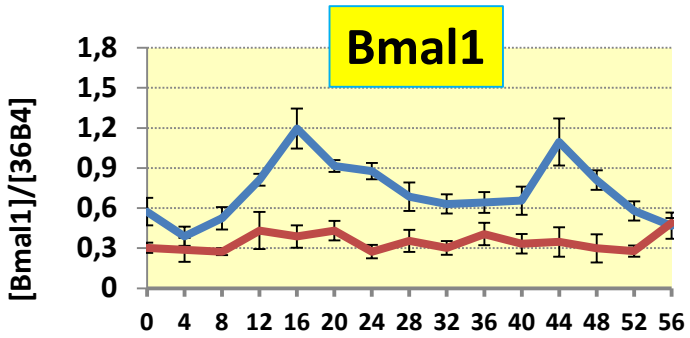
Clock-controlled irinotecan bioactivation and detoxification
Human CaCo-2 cells at confluence



Circadian Time since serum shock onset (h)

Control siRNA — *Bmal1 siRNA*

Cells exposed to siRNA then synchronized 24 h later with a 2h 50% FCS shock

Dulong et al. Submitted

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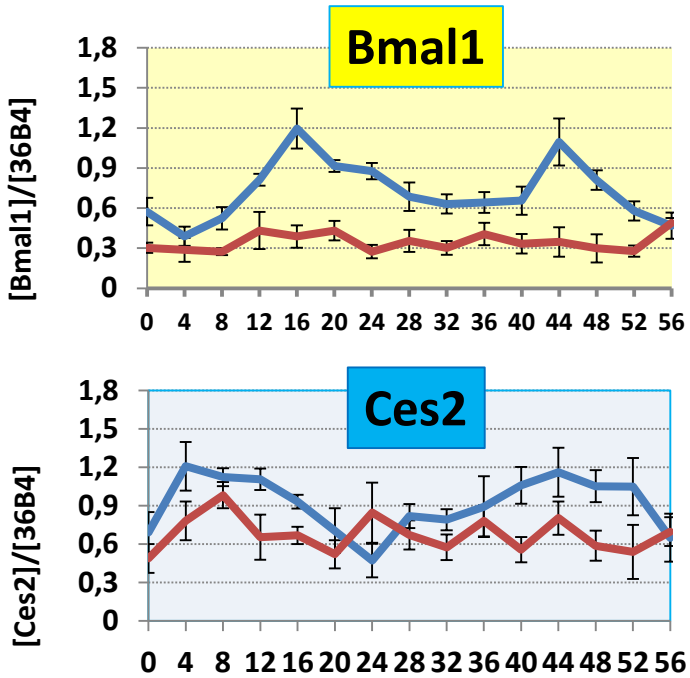

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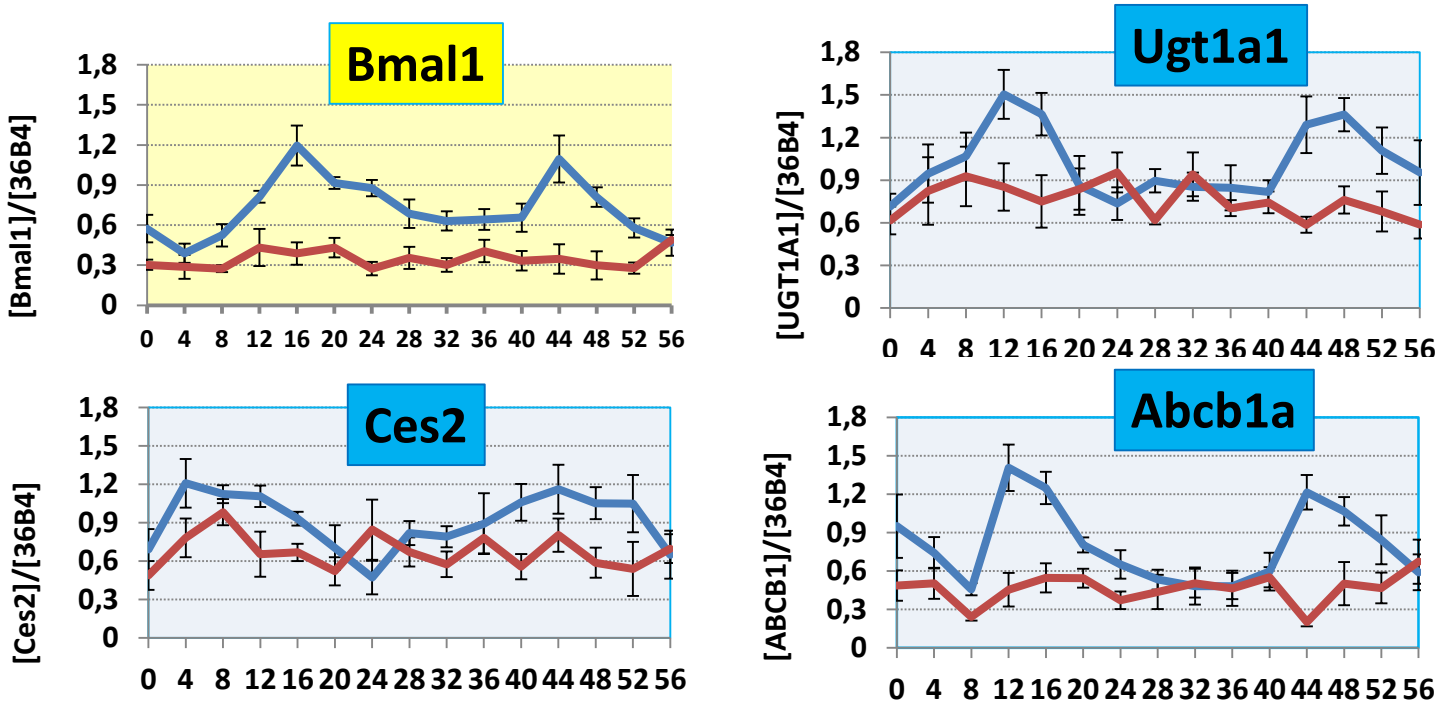

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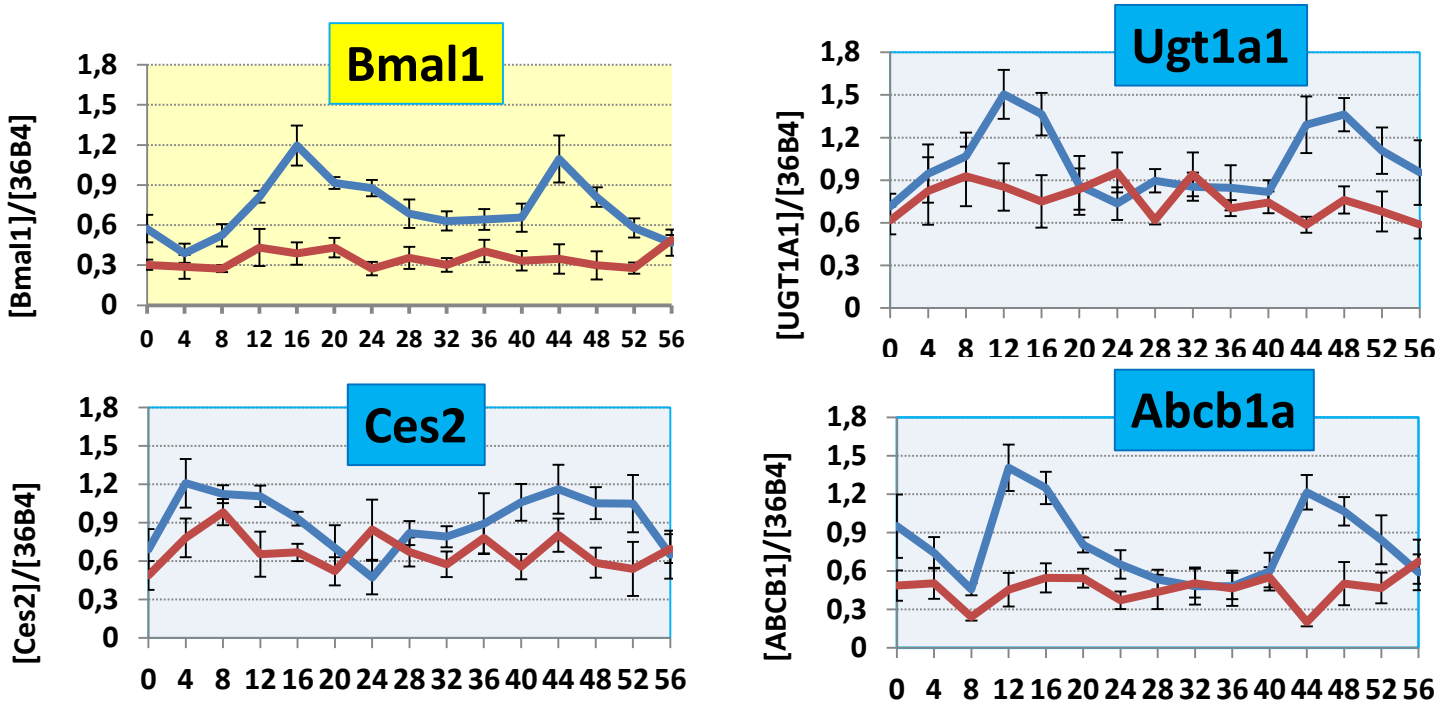

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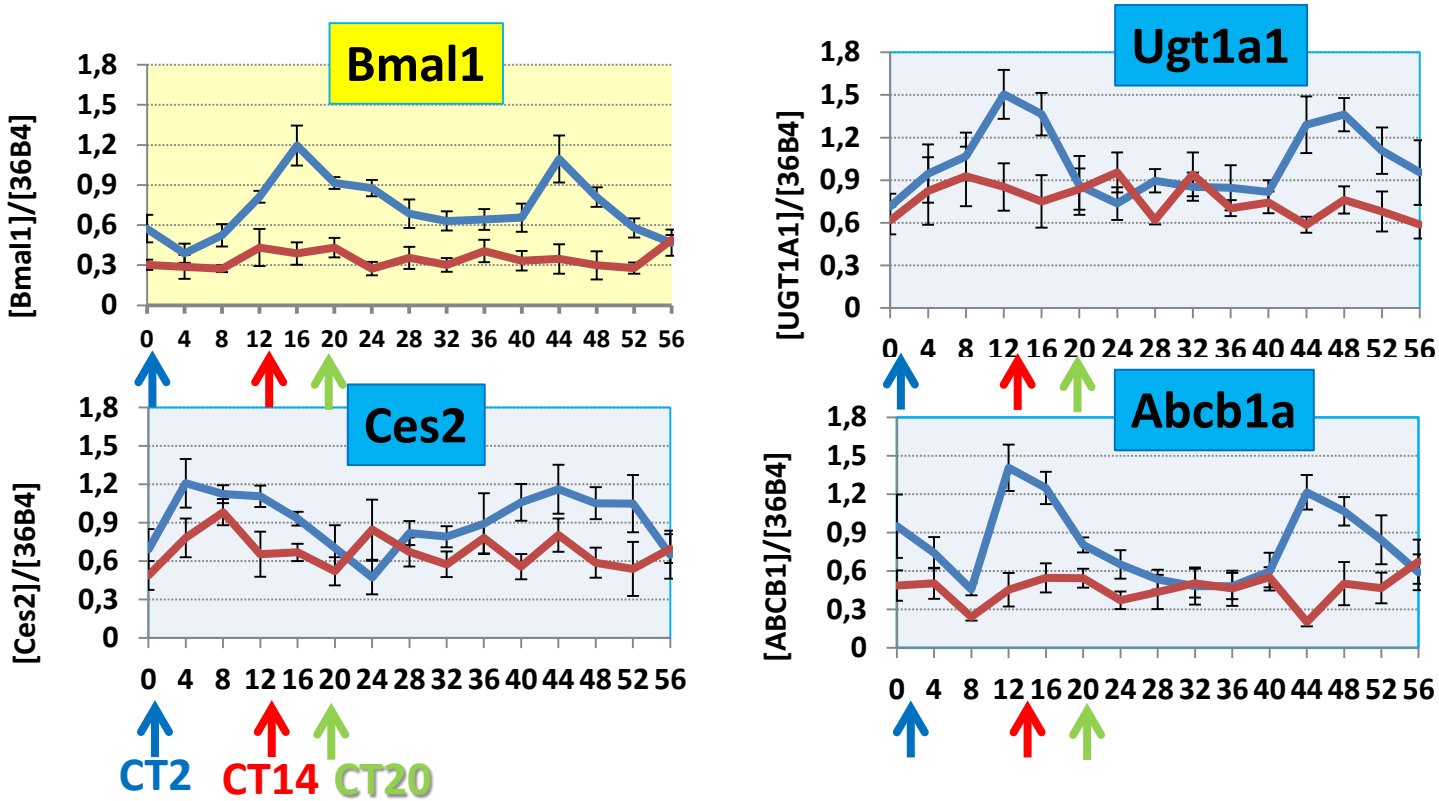

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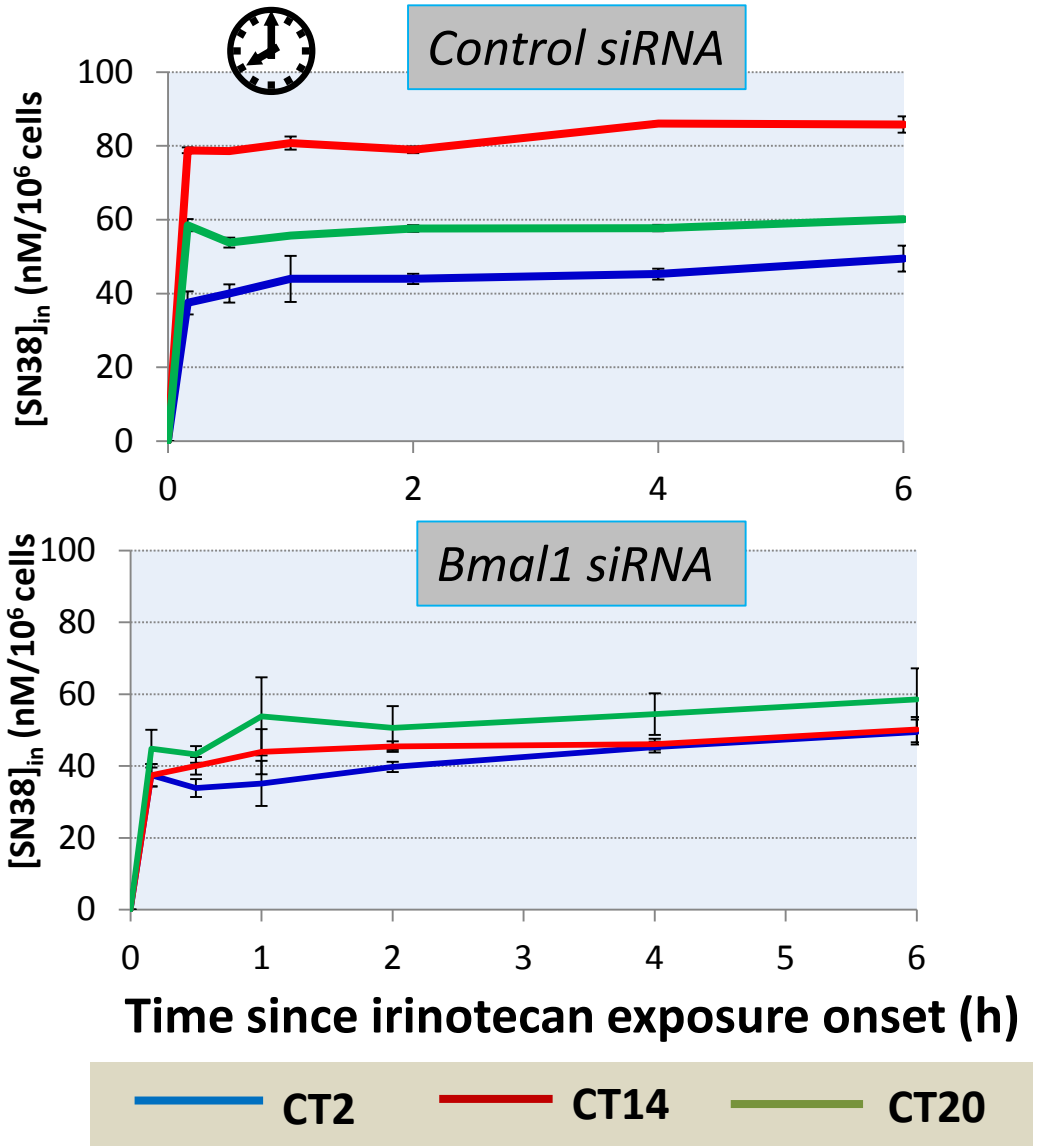

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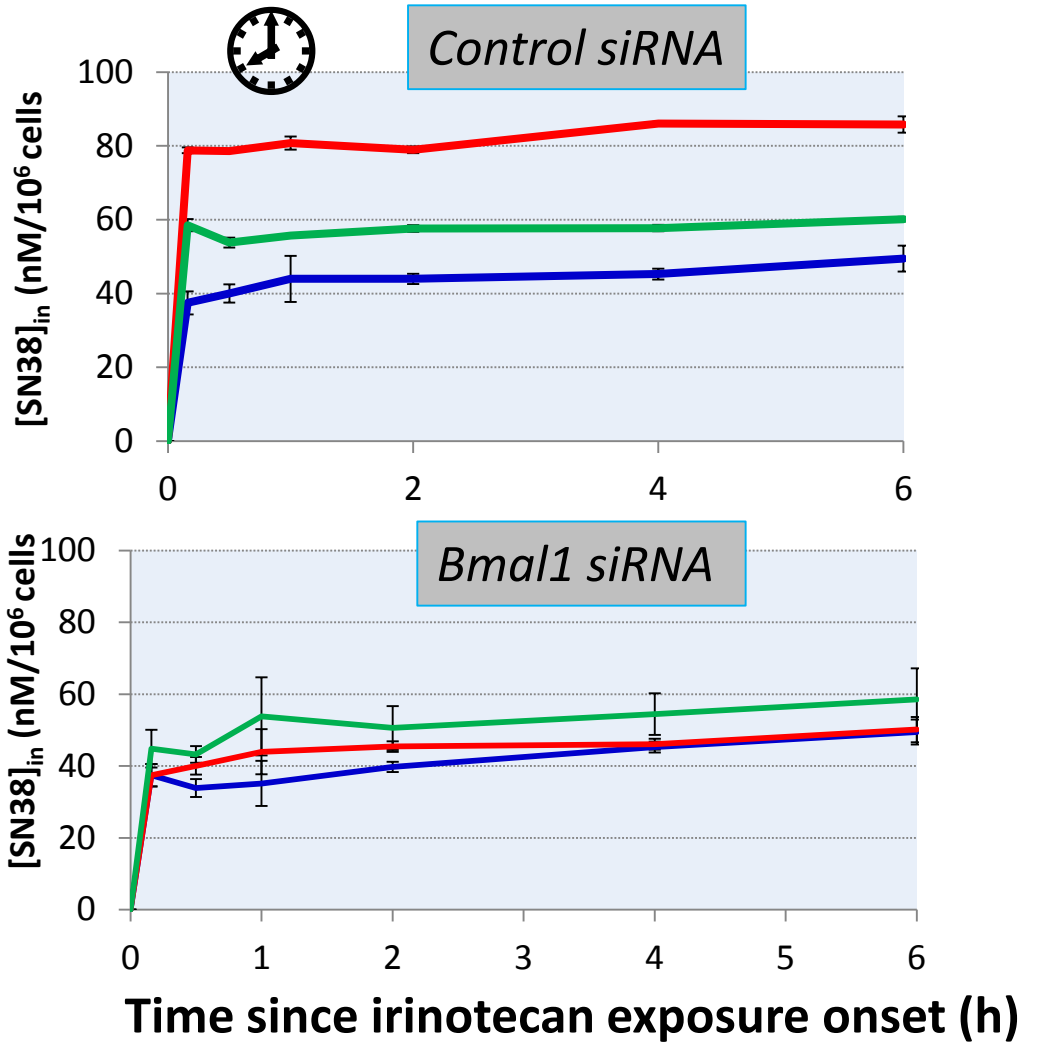
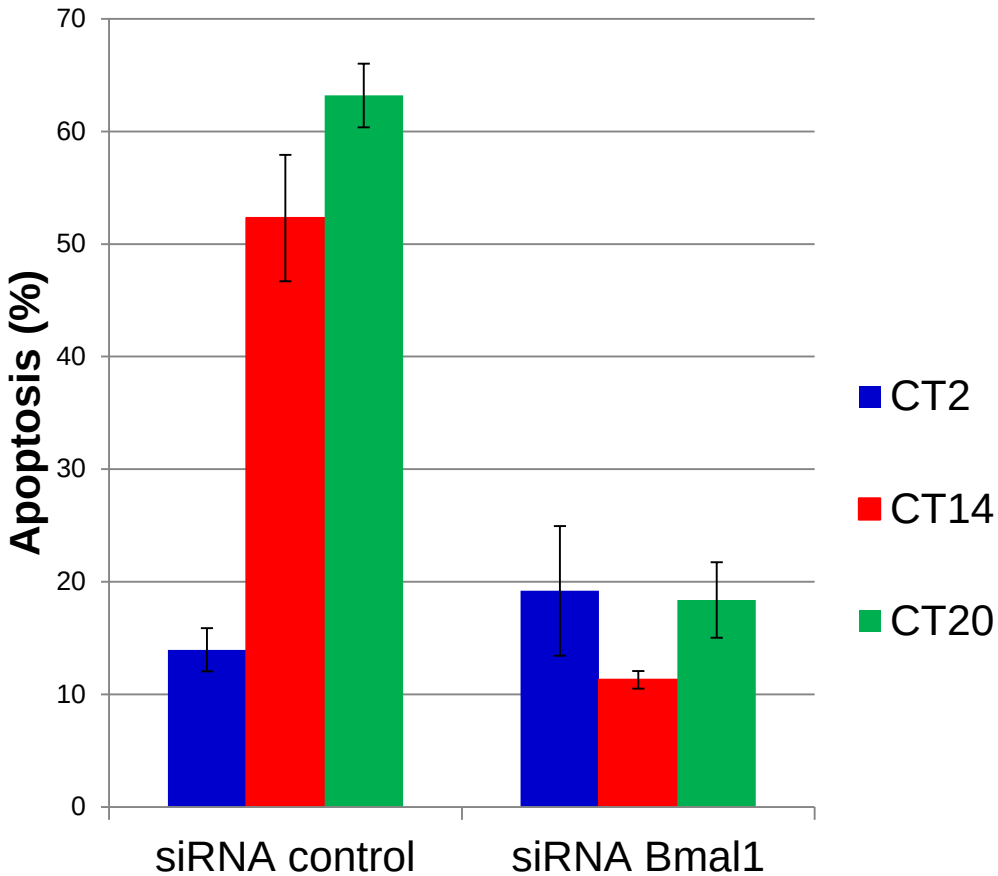




Dulong et al. Submitted

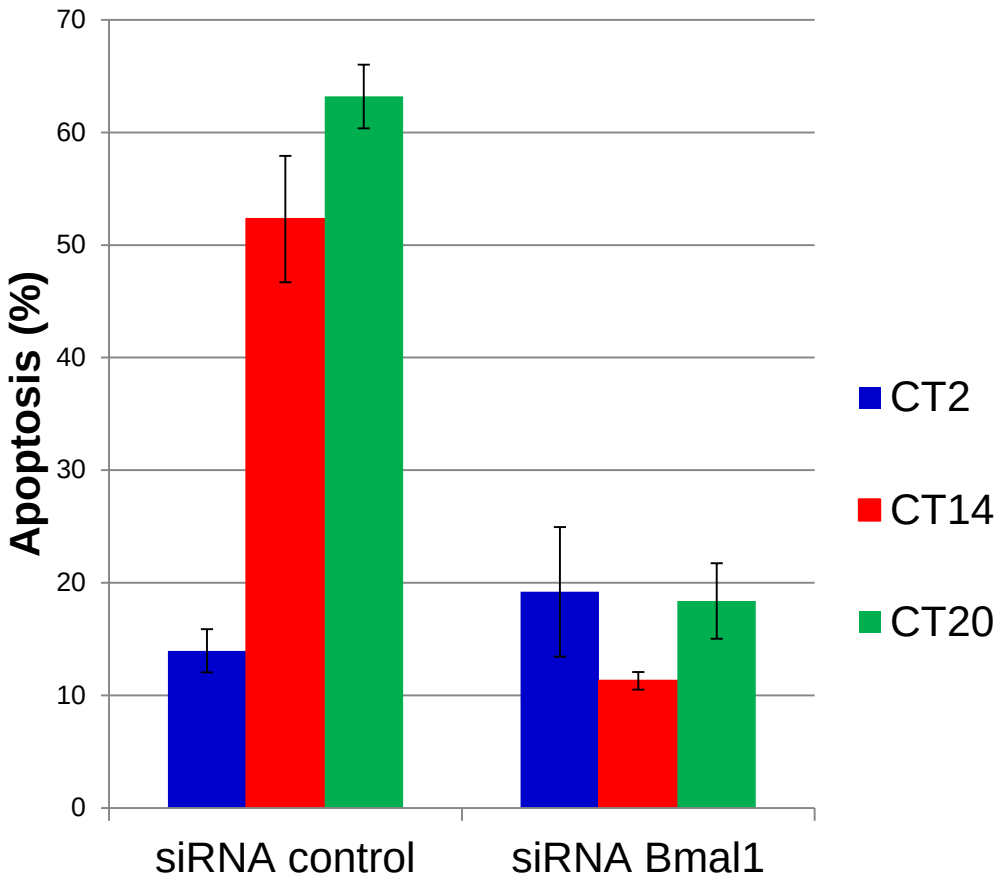
Clock-controlled irinotecan bioactivation and cytotoxicity (apoptosis)

Model and sensitivity analyses

- confirm critical role for clock control of CES2 irinotecan bioactivation
- add critical role for clock control of UGT1A1 irinotecan detoxification

Model validation in

- other cell lines (ongoing)
- mice (ongoing)
- cancer patients (circadian biomarkers)

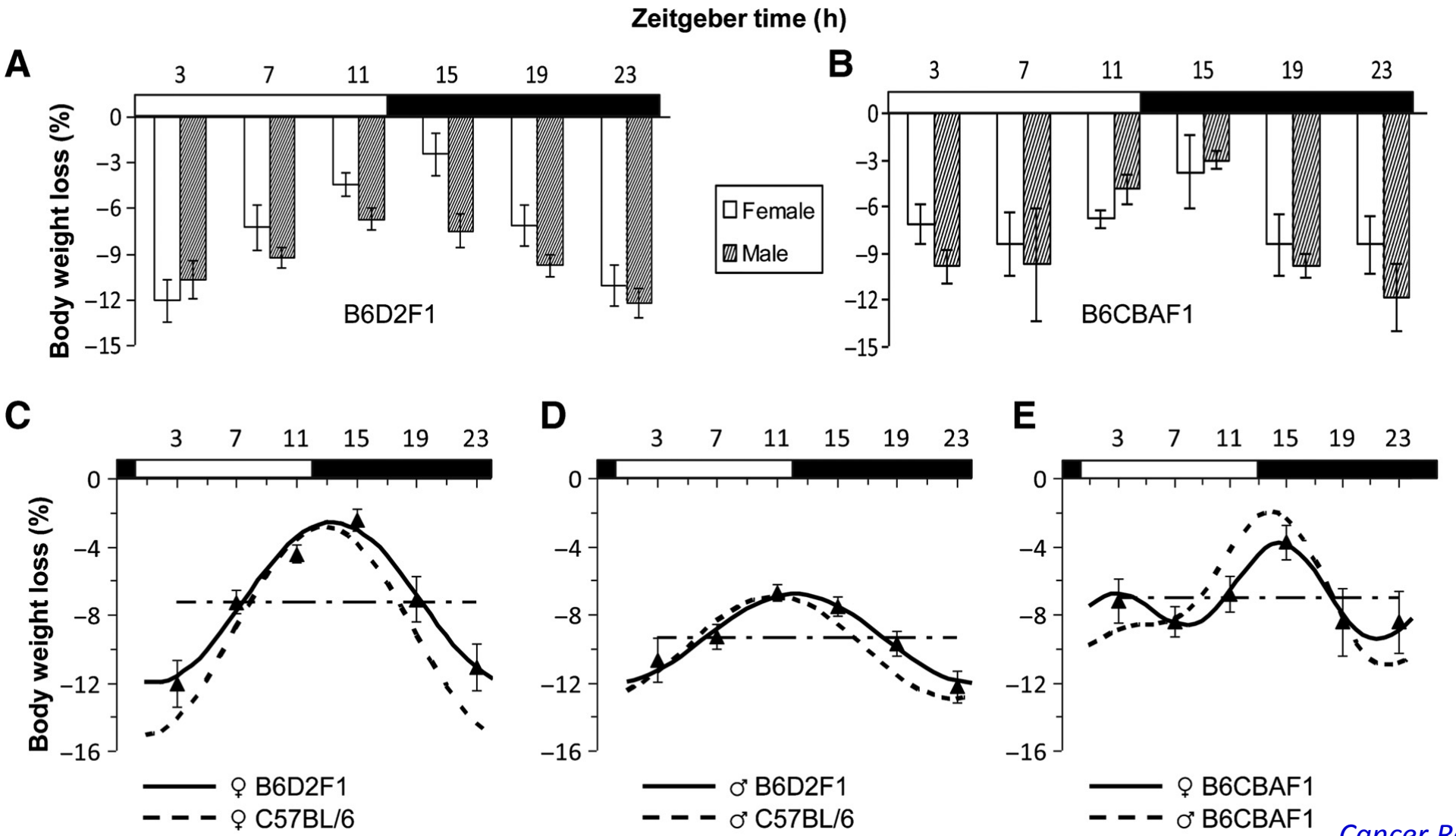


Dulong et al. Submitted

Clock- Chronopharmacology at cell population level

- Non malignant & cancer cells other than CaCo-2 at confluence?
- Impact of clock-cell cycle coupling?
- Circadian synchronisation?
- Robust chronotherapy model linked to clock markers?
- *In vivo* validation?

Sex and genetic differences in irinotecan chronotolerance in mice : 3 classes



Li et al.
Cancer Res 2013

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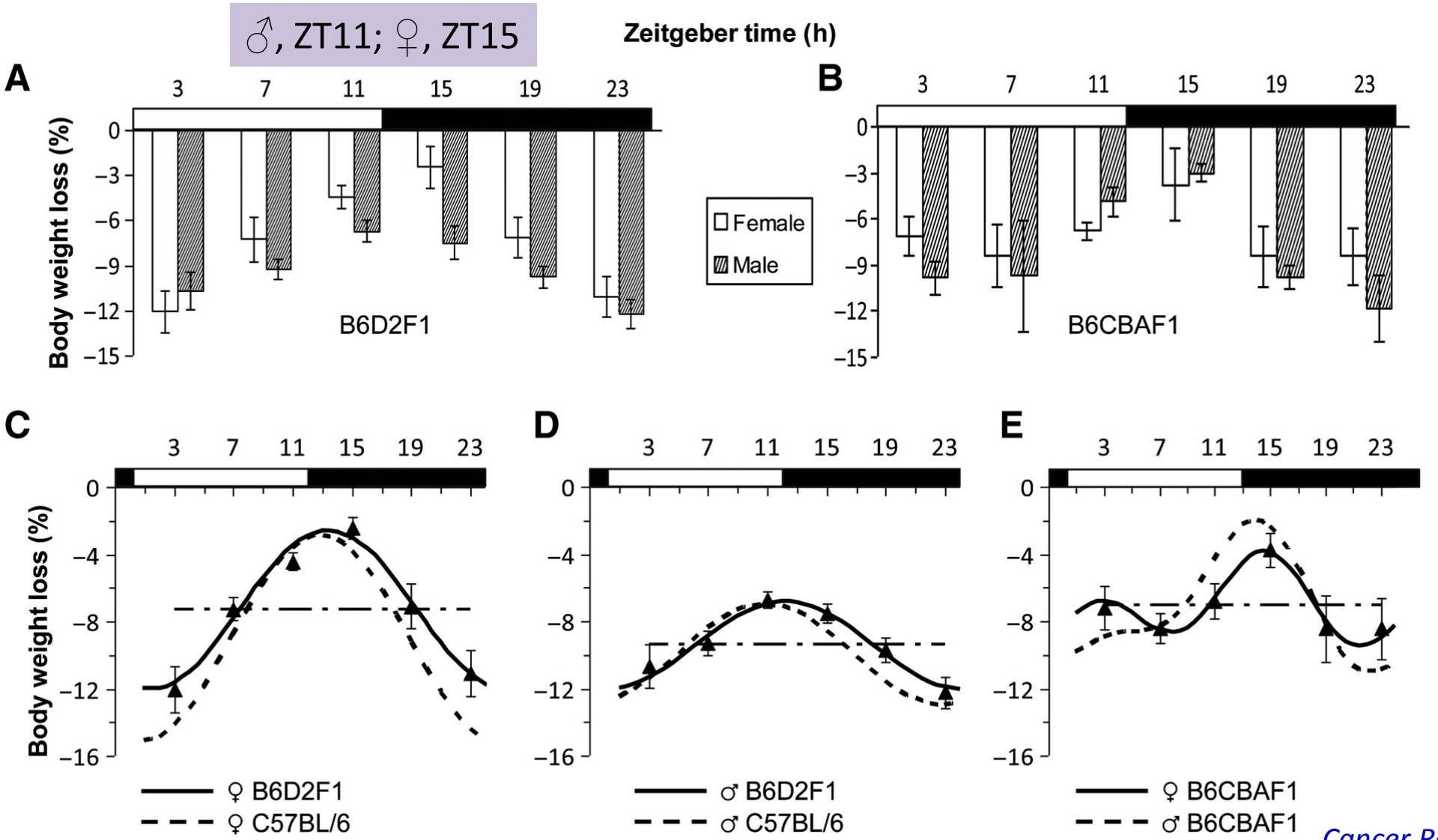

Li et al. Cancer Res 2013

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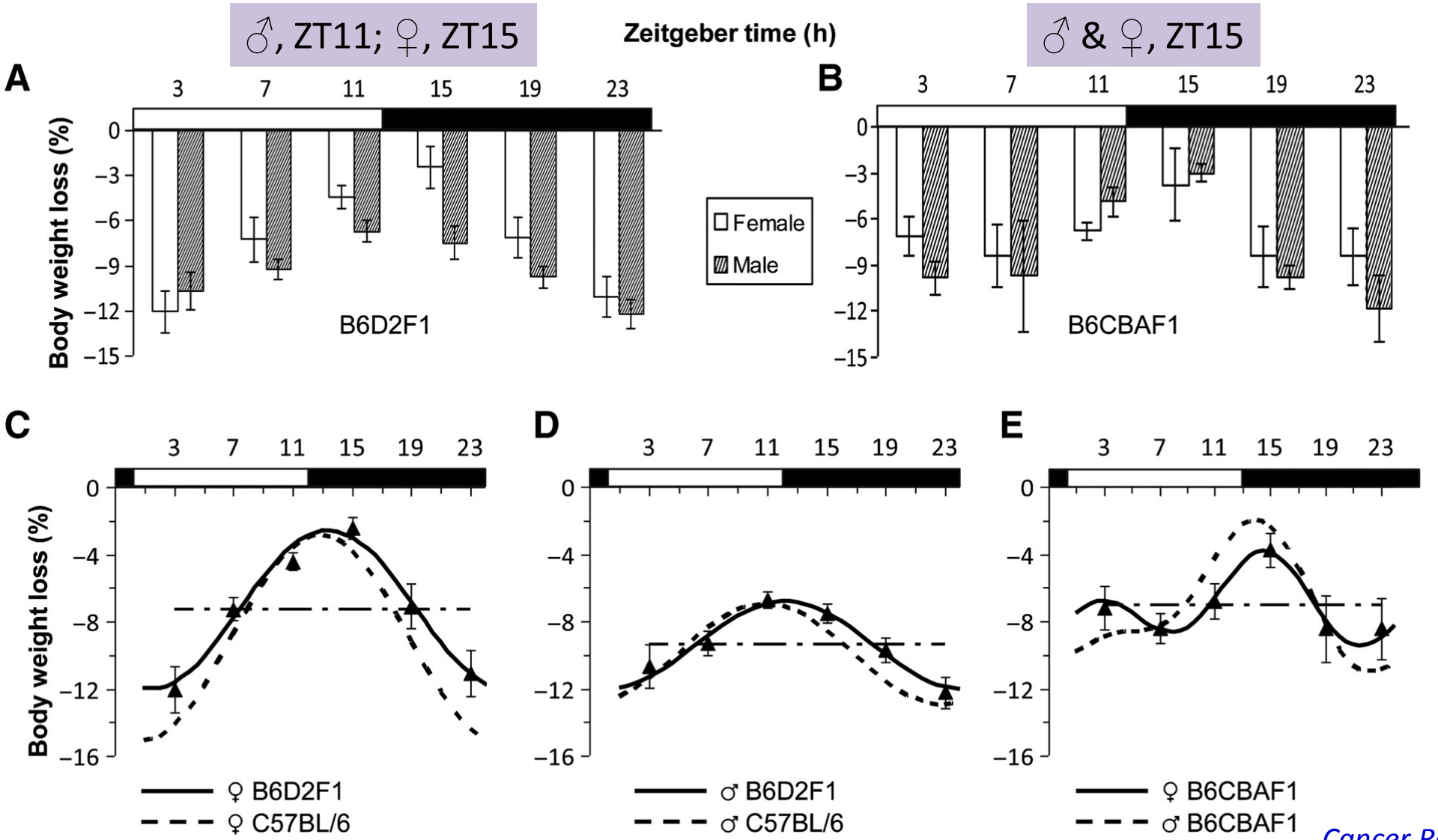
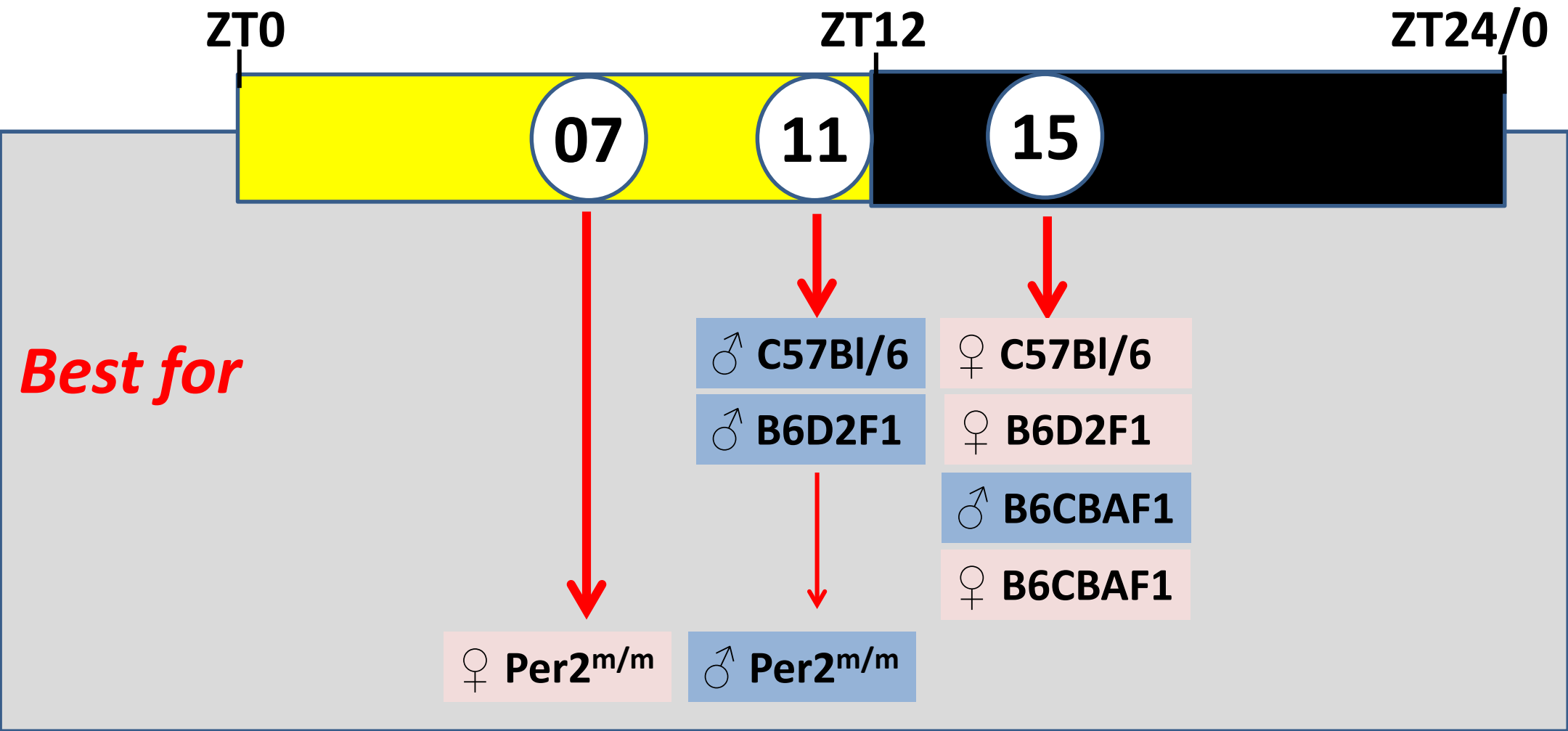
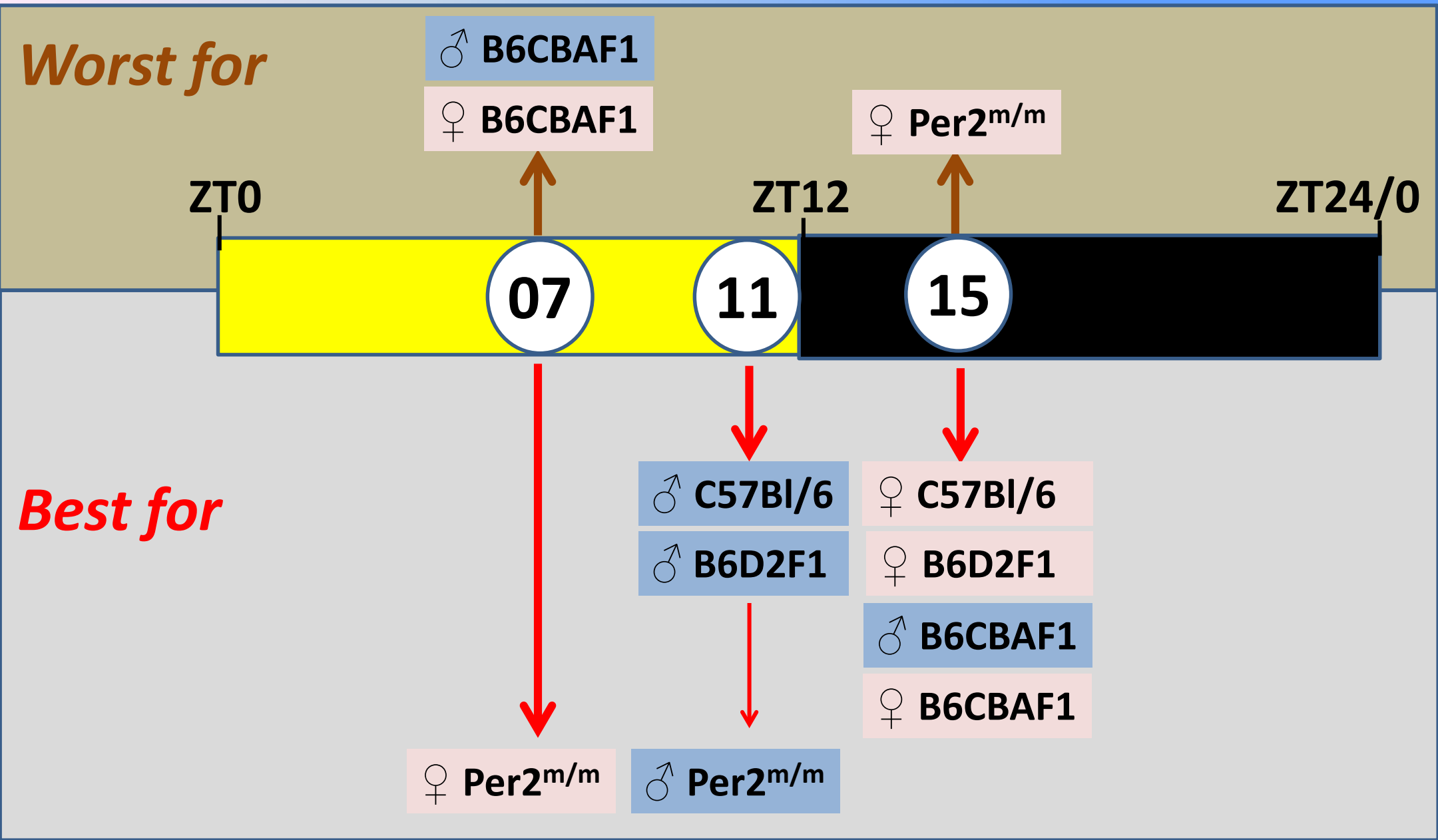
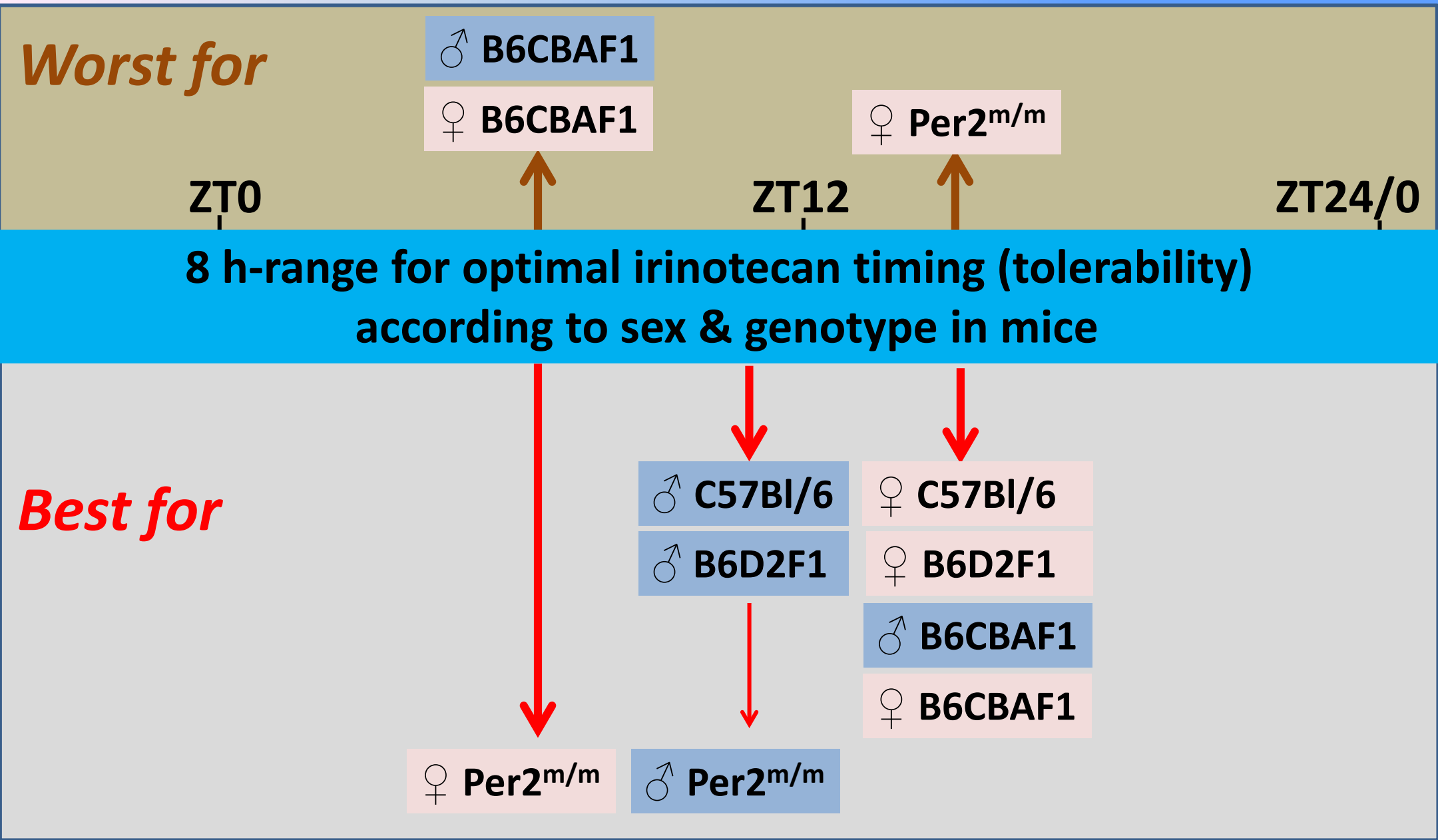


Li et al. Cancer Res 2013


ZT0
ZT12
ZT24/0
07
11
15
Best for
♂ C57Bl/6
♂ B6D2F1
♀ C57Bl/6
♀ B6D2F1
♂ B6CBAF1
♀ B6CBAF1
♀ Per2m/m
♂ Per2m/m

Systems Cancer Chronotherapeutics




Worst for
♂ B6CBAF1
♀ B6CBAF1
♀ Per2m/m
ZT0
ZT12
ZT24/0
8 h-range for optimal irinotecan timing (tolerability)
according to sex & genotype in mice
Best for
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8 h-range for optimal irinotecan timing (tolerability) according to sex & genotype in mice

Which of 27 liver or colon circadian gene transcription patterns predict for optimal timing if any?

Li et al. Cancer Res 2013

Systems Cancer Chronotherapeutics

Xiao Mei Li

V Hossard

M Dumitru

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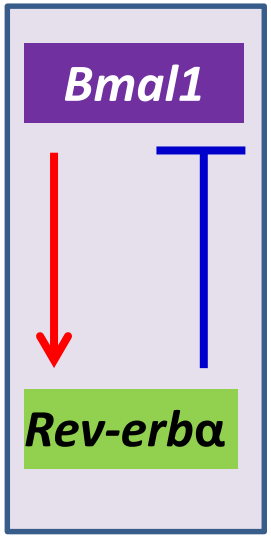

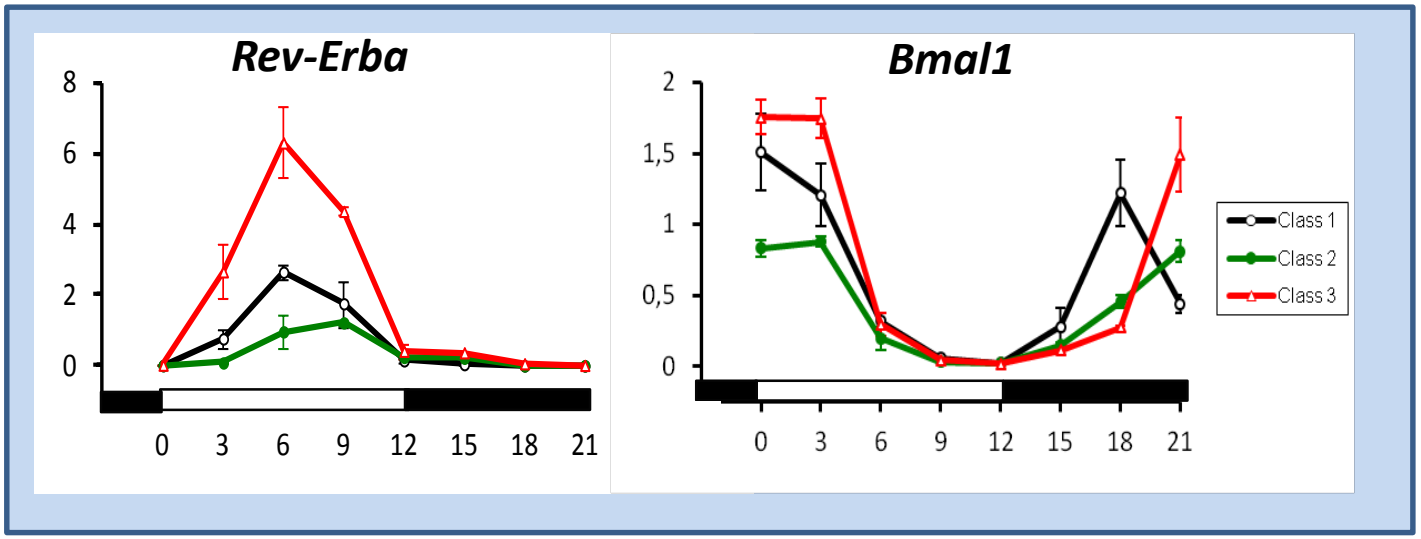

M Dumitru

Li et al. Cancer Res 2013

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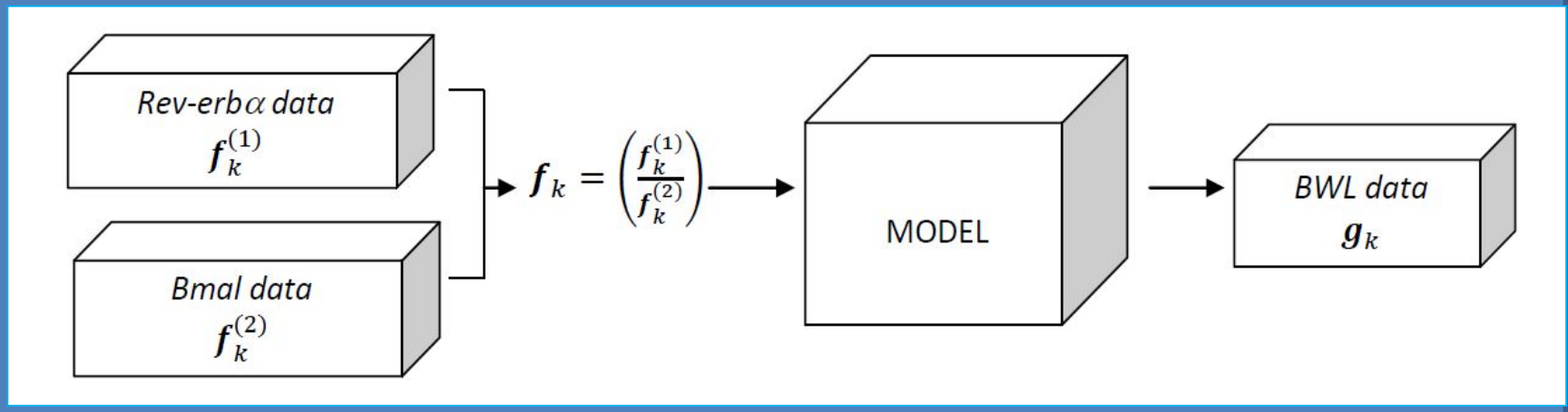
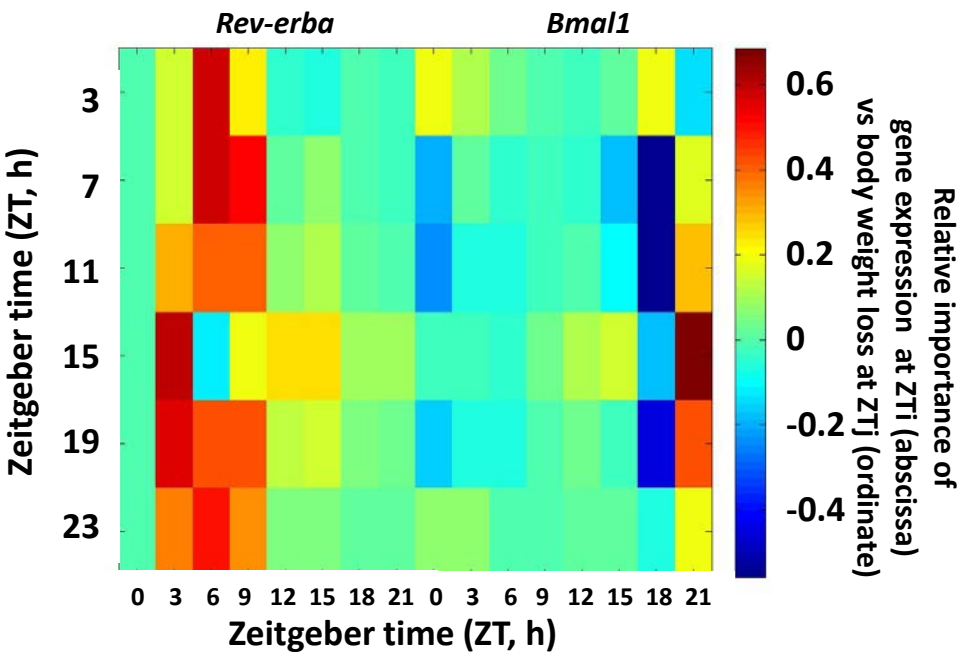
Ali Mohammad-Djafari (CNRS, SUPELEC, Paris Sud Univ)

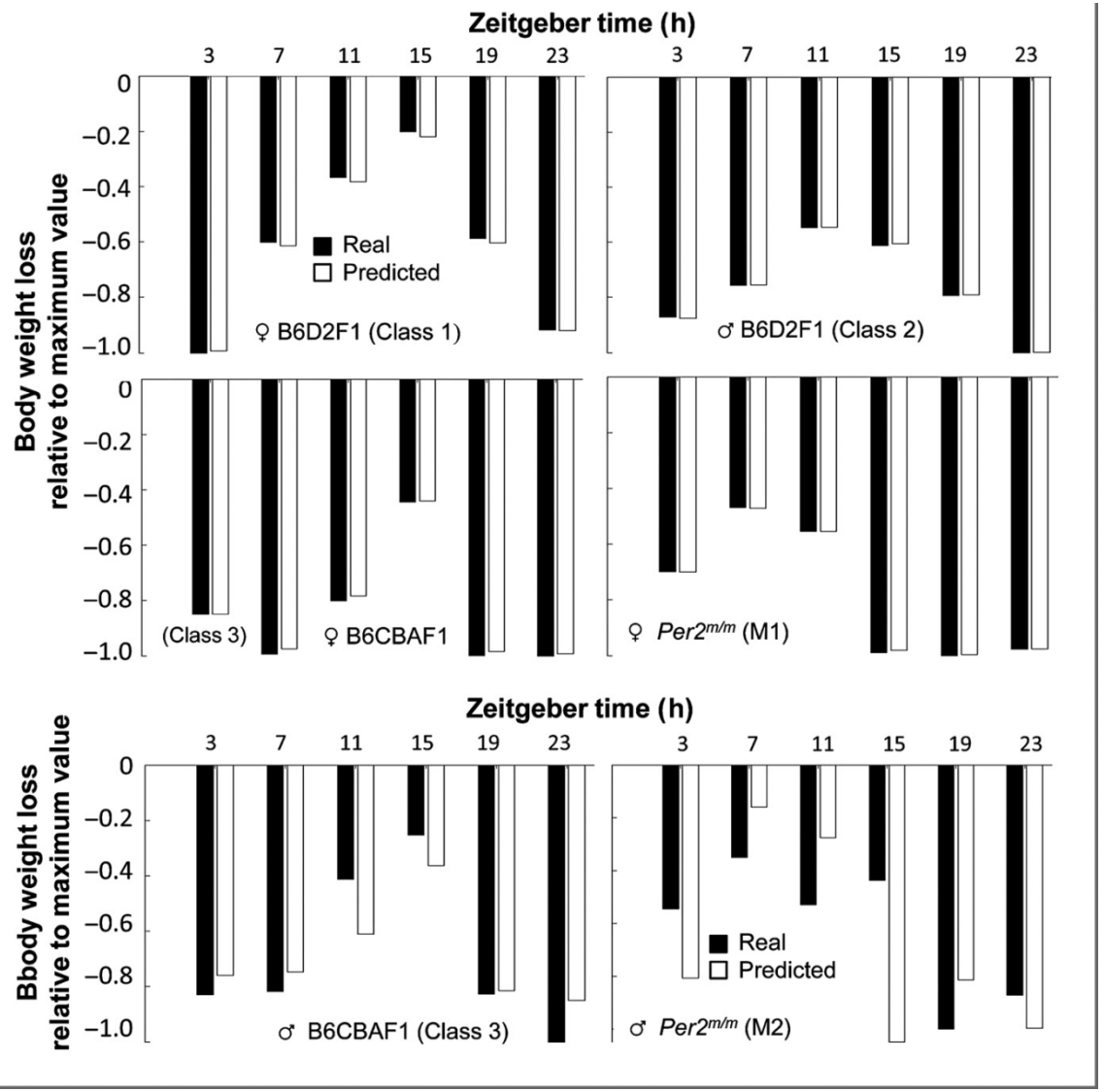


A circadian clock transcription model for the personalization of cancer chronotherapy timing

Li et al. Cancer Res 2013

A circadian clock transcription model for the personalization of cancer chronotherapy





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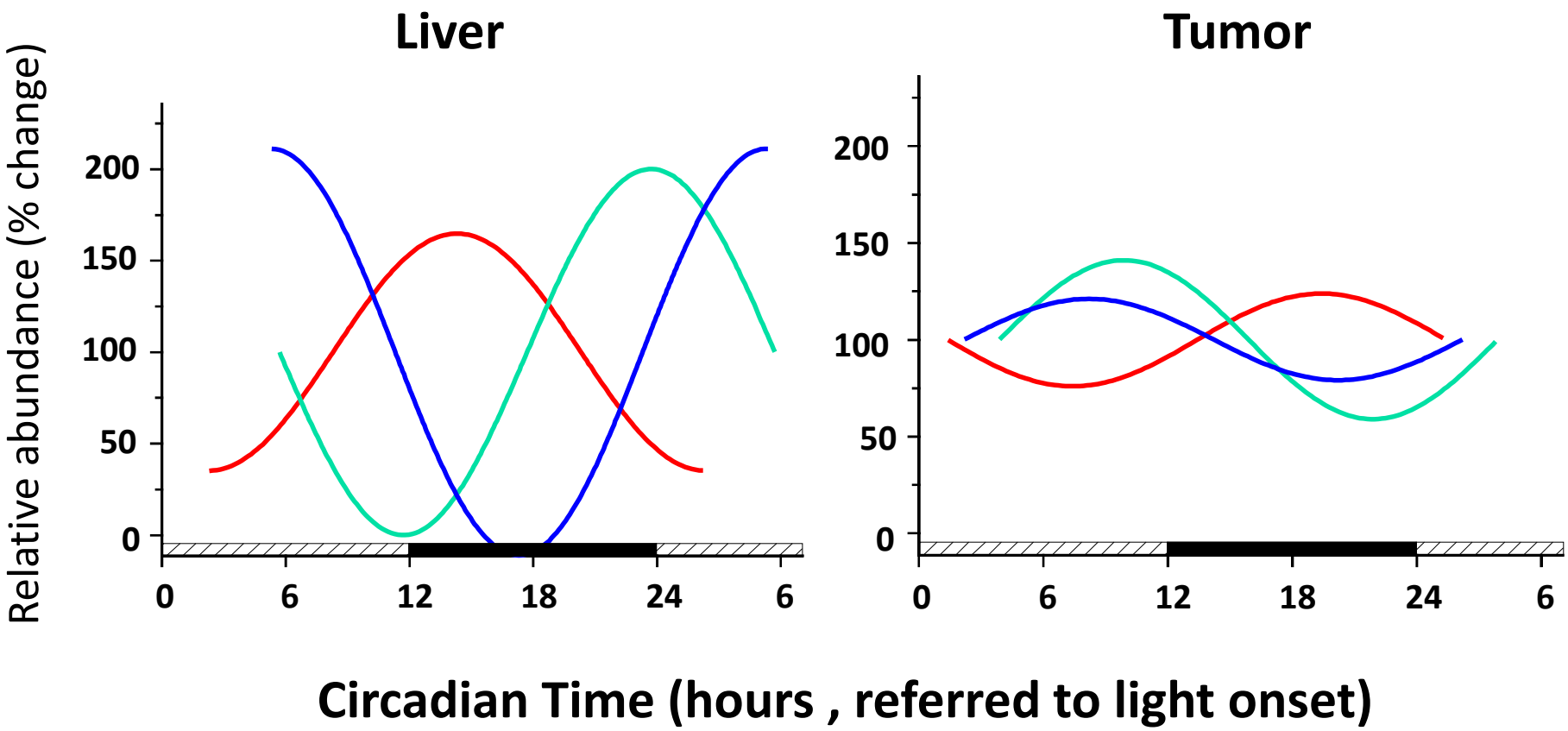
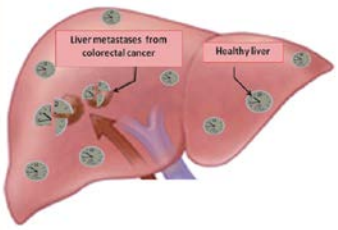


mRNA transcription

Rev-erbα — Per2 — BMal1

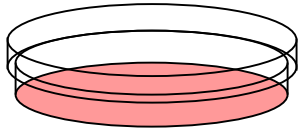
Circadian Control of Cancerogenesis & Cancer Chronopharmacology

Circadian Control of Cancerogenesis & Cancer Chronopharmacology



- **Systematic exploration of role of clock genes in liver and cancers for cell cycle, drug metabolism and chronotherapeutic effects**
- **Continuous physiology monitoring & dynamic molecular imaging**

Per2::luc/Bmal1::luc
± si/shRNA
Rev-Erbα-FUCCI



Lumicycle

Per2::luc
Abcb1a::luc

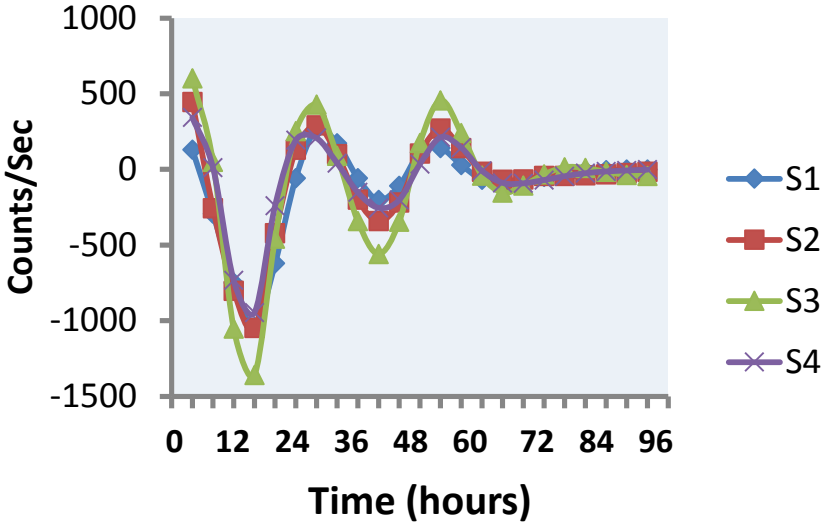
Clock mutant
Cancer prone

RT-Bio

IVIS Spectrum

Per2 trancription dynamics in liver cancer (HEPA1-6 Per2::luc clone3)

Dex-pulsed cultures (40% confluence)



XM Li, V Roche, N Ozturk, M Dumitru, S Dulong

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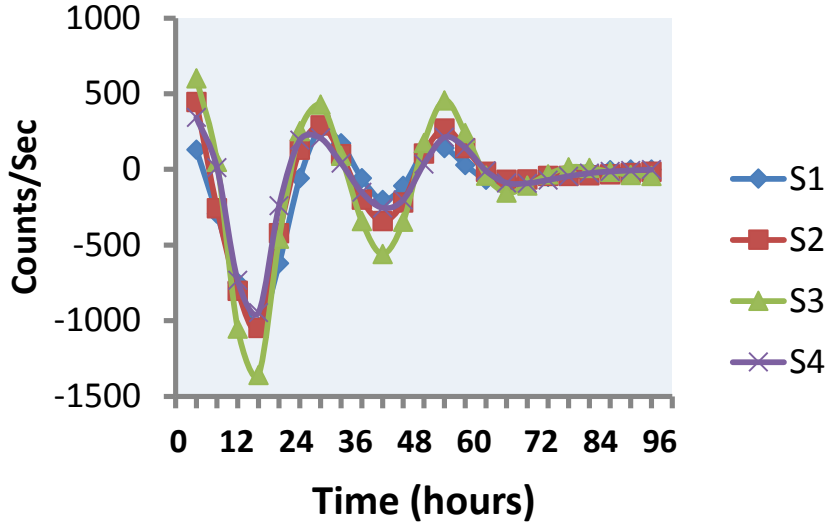
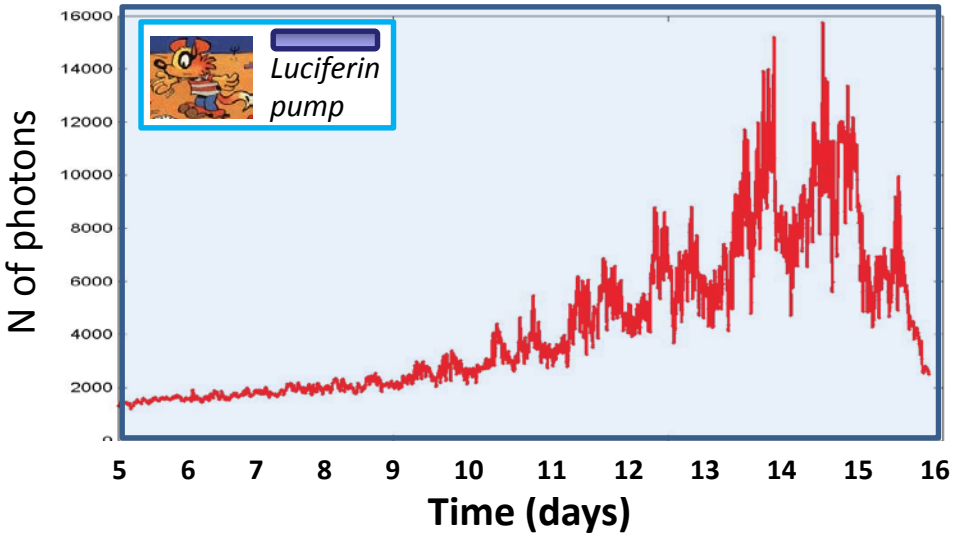


10^7 cells *sc on day 0 in NMRI mouse*



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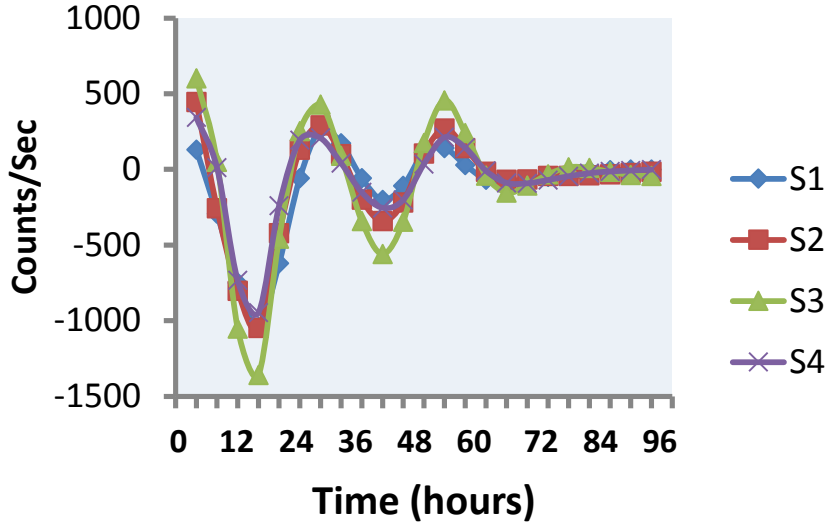

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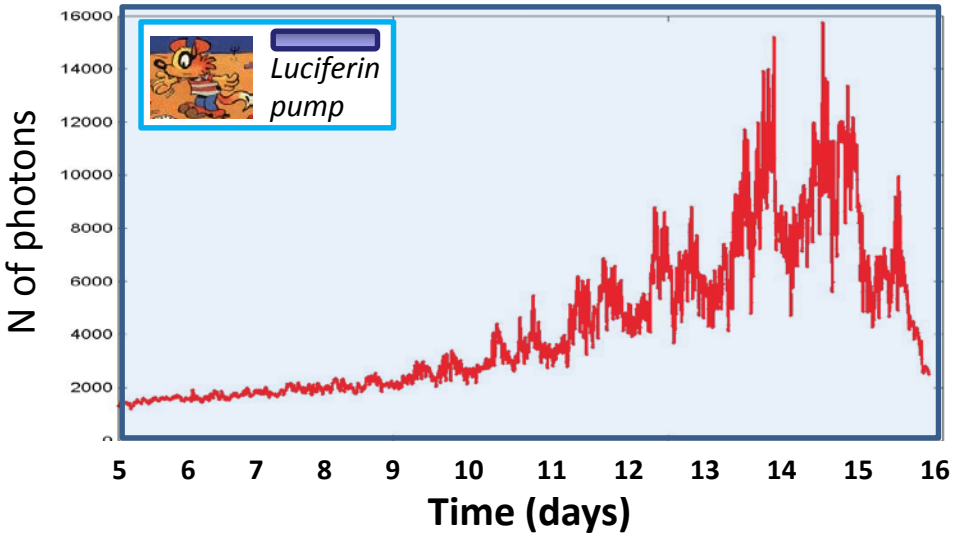
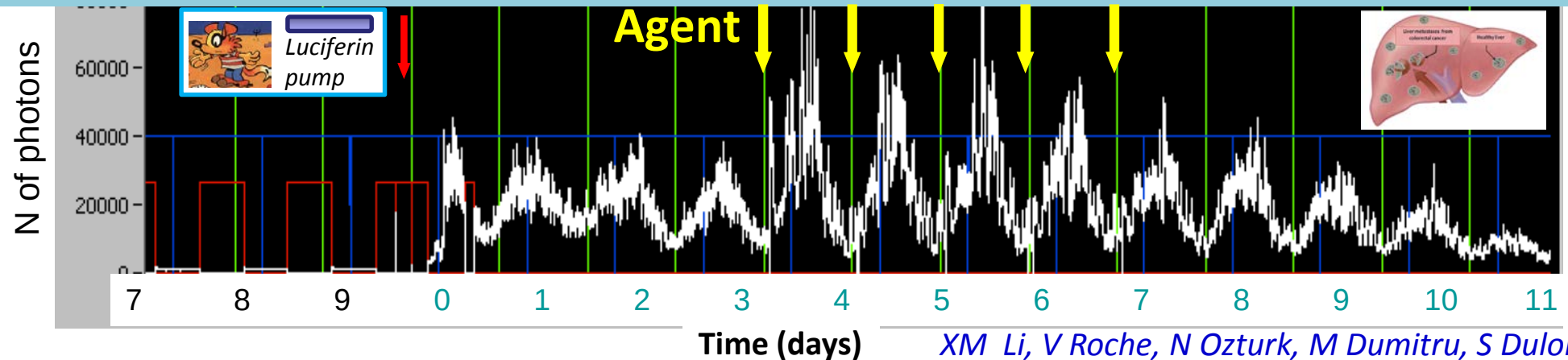


Per2 transcription dynamics in mouse liver (Per2::luc KI/KI mouse)



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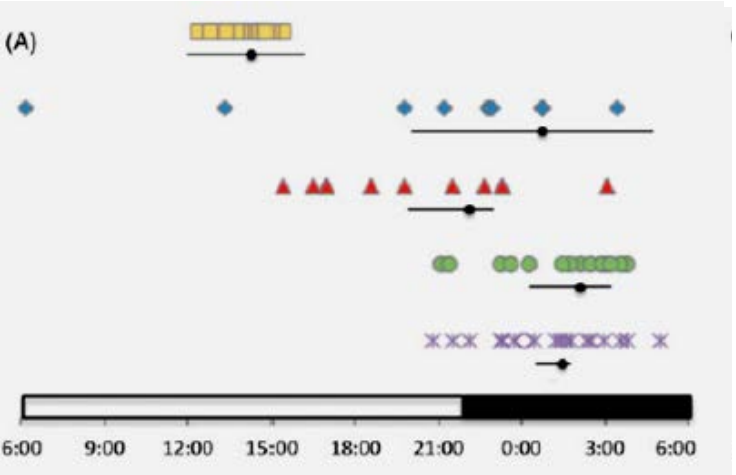
Timing of circadian maximum (acrophase) in rest-activity and skin surface temperature rhythms

10 individual patients before, during and after a 4-day course of chronotherapy

Before



Time (clock hours)

Rest-activity ***Temperature patch*** ◆ ***#1*** ▲ ***#2*** ● ***#3*** ✱ ***#4***

Roche et al. Chronobiology Intern 2014

Timing of circadian maximum (acrophase) in rest-activity and skin surface temperature rhythms

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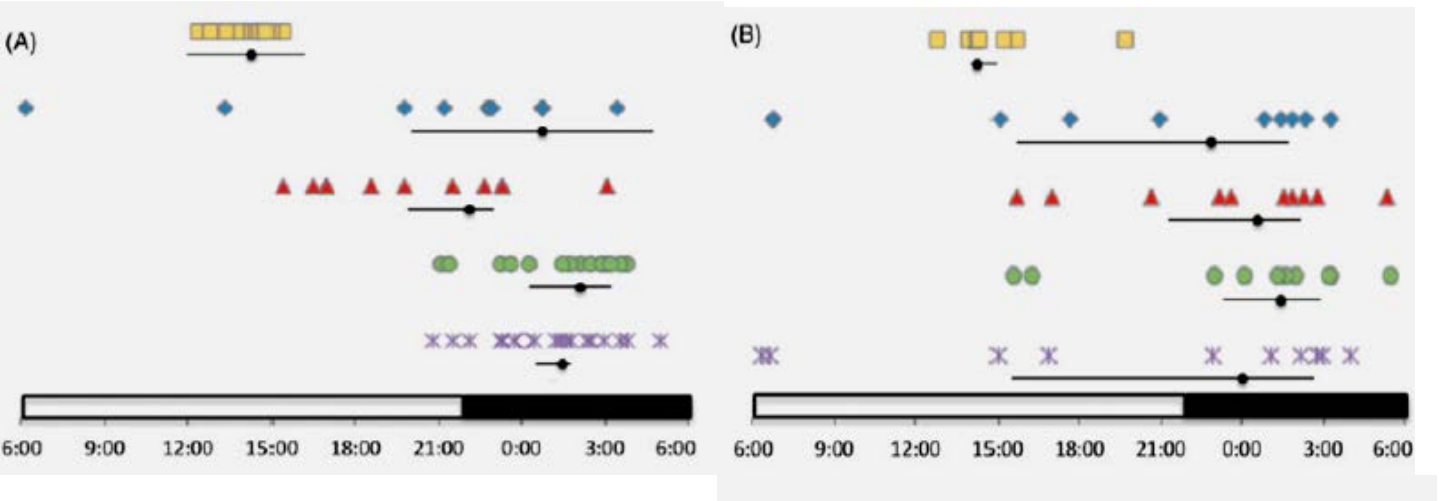
Before ***During***



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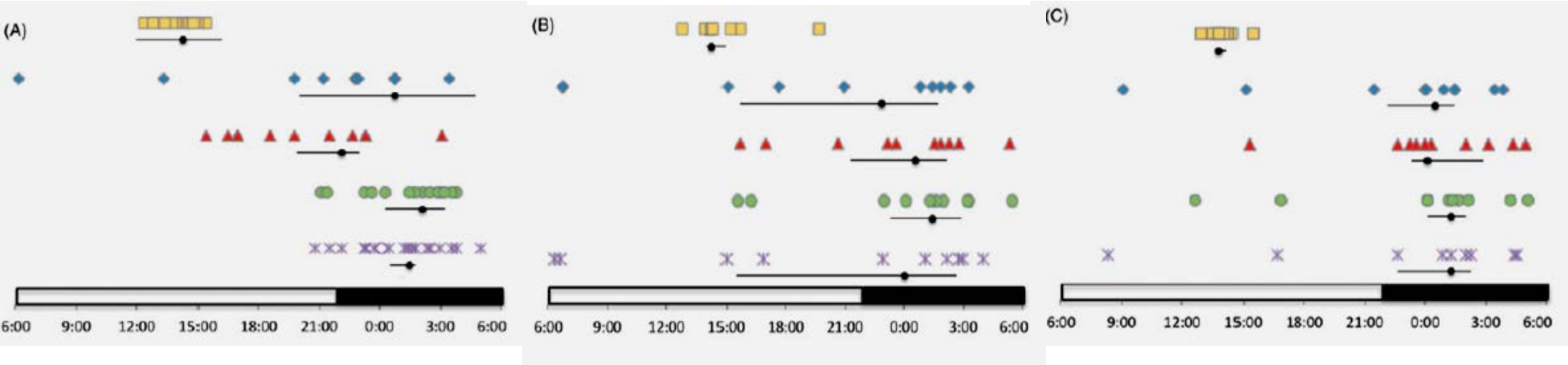

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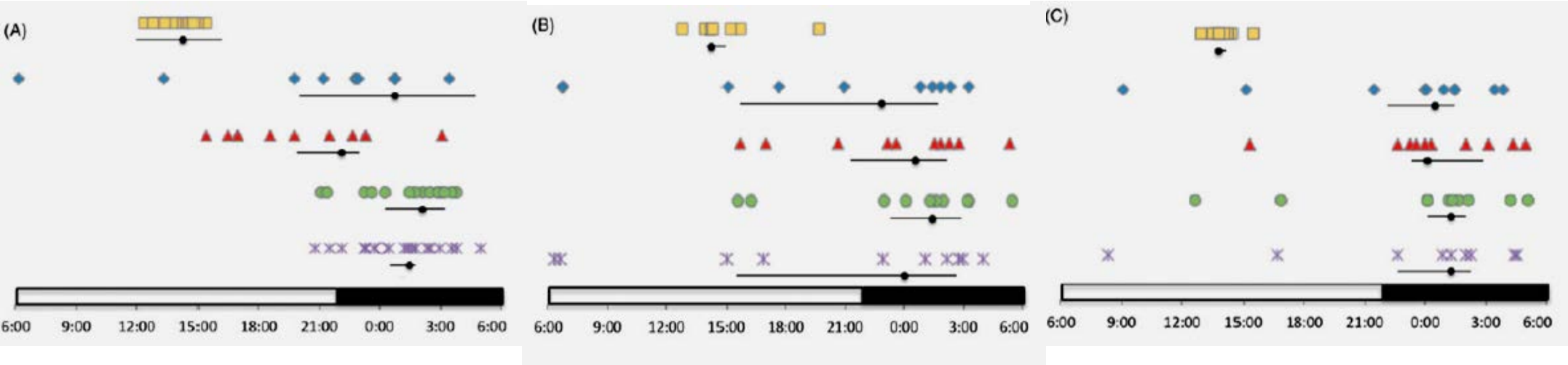

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Roche et al. Chronobiology Intern 2014

Conclusions

- The Circadian Timing System controls cancer processes and treatment effects through redundant coordinated mechanisms

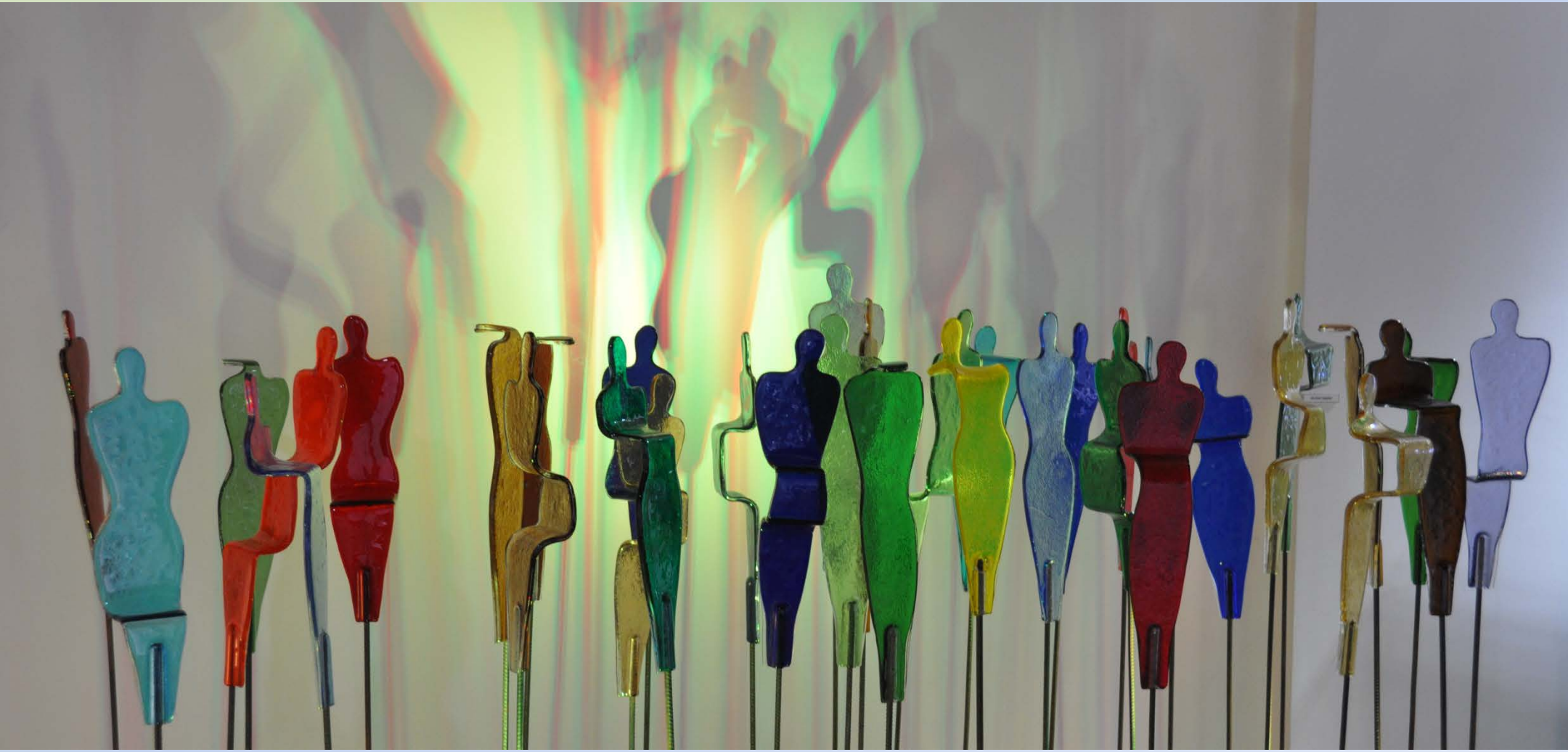
  → Targeting host clocks can prevent or halt cancer via host mechanisms

  → Targeting tumor clocks could enhance efficacy or cure cancer

- New coordinated *in vitro-in silico-in vivo* cancer chronotherapeutics (Systems Biology - Systems Medicine)

- Mathematical models and dedicated technologies critical for designing & delivering optimal chronotherapy schedule and strategy.

Circadian Control of Cancerogenesis & Cancer Chronopharmacology



Circadian Control of Cancerogenesis & Cancer Chronopharmacology

Rythmes Biologiques et Cancers

Cancersensor ANR-TECSAN

OPTILIV EUDRACT 2007-00463224