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Supplementary Table 1. A comparison of protein sequence identity/similarity, for several clock-relevant genes, between *Ostreococcus tauri* and *Homo sapiens*.

Supplementary Table 2. A summary of literature reports in other taxa for the reported targets of drugs that modulate free-running period in *Ostreococcus tauri*.

Supplementary Figure 1. Representative plots of circadian rhythms of gene expression in *O. tauri* following constant darkness.

Supplementary Figure 2. Sequence, expression and post-translational modification of PRX in *O. tauri*.

Supplementary Figure 3. The effects of inhibition of cellular RNA synthesis or cytosolic translation upon transcriptional and translational bioluminescent clock reporters under constant light.

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Supplementary Figure 5. Representative and grouped data showing similar pharmacological actions on circadian period in *O. tauri* to those reported in other taxa.

Supplementary Figure 6. Representative and grouped data showing the effects of pharmacological modulators of circadian period in *O. tauri*, in the absence of transcription.

O'Neill *et al*, Supplementary Table 1

Putative *O. tauri* drug target	Accession	Closest *H. sapiens* homologue	Accession	Sequence Identity (%)	Sequence Similarity (%)	E-value (NCBI Blast, BLOSSUM 62)
DNA topoisomerase II	CAL56339	DNA topoisomerase II	AAA61209.1	54	70	0
HSP90	CAL56087	HSP90	NP_005339.3	67	85	6.00E-170
PP2A	CAL51458.1	PP2A	NP_060931.2	51	62	4.00E-127
CK1	CAL52491	CK1	NP_001884.2	72	82	2.00E-126
GSK3	CAL51449.1	GSK3	NP_001139628.1	60	74	6.00E-125
MAPK	CAL55559.1	MAPK	NP_620407.1	54	71	6.00E-103
CK2	CAL52182	CK2	CAI18393.2	58	71	1.00E-62
Proteasome beta subunit	CAL50436	Proteasome subunit	NP_002786.2	50	67	8.00E-51
Adenylyl cyclase	CAL54153	No relevant hits[1]	-	-	-	-

O. tauri clock gene	Accession	Closest H. sapiens homologue	Accession	Sequence Identity (%)	Sequence Similarity (%)	E-value (NCBI Blast, BLOSSUM 62)
CCA1	AAU14271	MYB-like[2]	BAB67808.1	38	75	2.00E-05
TOC1	AAU14274	No relevant hits	-	-	-	-

H. sapiens clock gene	Accession	Closest *O.tauri* homologue	Accession	Sequence Identity (%)	Sequence Similarity (%)	E-value (NCBI Blast, BLOSSUM 62)
Period2	ABM64216	DNA repair and transcription factor XPB1[3]	CAL53063.1	25	40	0.3
Bmal1b	BAA19935	Unnamed protein product[3]	CAL56979.1	36	56	0.18

Table S1. A comparison of protein sequence identity/similarity, for several clock-relevant genes, between *Ostreococcus tauri* and *Homo sapiens*.

[1] *O. tauri* encodes two proteins (accessions: CAL50189.1, CAL54153.1) that are annotated as members of the class III nucleotidyl cyclase superfamily. These have little significant sequence homology with mammalian adenylyl cyclases (also class III). Due to the mode of action of 9-(Tetrahydro-2-furanyl)-9H-purin-6-amine, 9-THF-Ade (THFA) - a post-transition state, non-competitive p-site ligand that binds the catalytic site by mimicking cAMP, it is plausible that this drug is preferentially active against adenylyl cyclase in *O. tauri* despite an clear divergence of primary protein sequence.

[2] Not implicated in the mammalian clockwork.

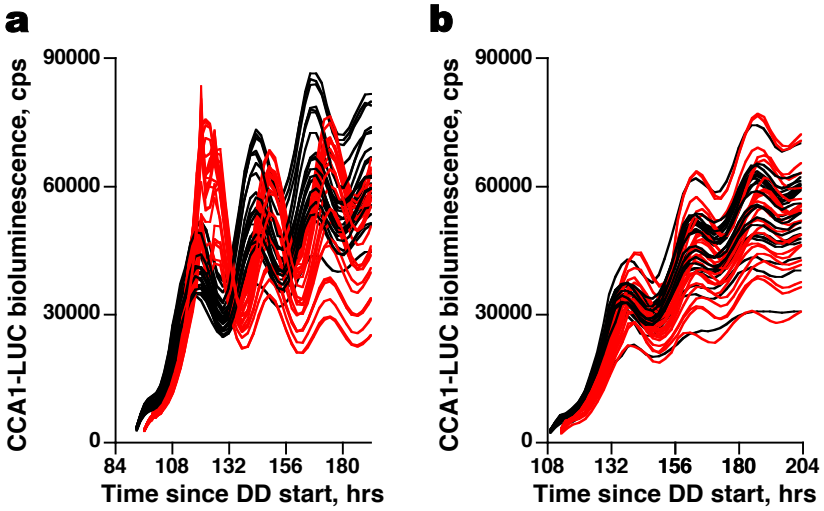
[3] Not implicated in the plant clockwork.

O'Neill *et al*, Supplementary Table 2

Compound	Target	Notes	Reference(s)
amsacrine-HCL	DNA topo II	Shown to shorten period in mammalian cells	Isojima et al, PNAS, 2009
SB216763	GSK3	Shown to shorten period in mammalian cells, GSK3 also implicated in the *Drosophila* clock	Isojima et al, PNAS, 2009 Hirota et al, PNAS, 2008
$LiCl_2$	GSK3	Increases period in a wide range of organisms, shown to act through GSK3 in mammalian cells	Iitaka et al, J Biol Chem, 2005 Yin et al, Science, 2006
IC261	CK1	Lengthens period in mammalian cells and in *Neurospora*	Eide et al, Mol Cell Biol, 2005; Querforth et al, Cold Spring Harb Symp Quant Bio, 2007;
D4476	CK1	Lengthens period in mammalian cells	Reischl et al, J Biol Rhythms, 2007
DMAT	CK2	Increases period in mammalian tissue *in vitro*.	Maier et al, Genes Dev, 2009 Mizoguchi et al, Int Rev Cytol, 2006
TBB	CK2	CK2 is also heavily implicated in *Arabidopsis*, *Neurospora* and *Drosophila* clock mechanisms	Miyata, Mol Cell Biol, 2004
nicotinamide	Ca^{2+} signaling &/ or NAD metabolism	Nicotinamide increases period in *Arabidopsis* and mammalian cells	Dodd et al, Science, 2007; Asher, Cell, 2008
BAPTA	Ca^{2+} signaling	Ca^{2+} signaling is also heavily implicated in *Drosophila* and mammalian clock mechanisms	Harrisingh et al, J Neurosci, 2007; Lundkvist et al, J Neurosci, 2005; Ikeda, Neuron, 2003
SP600125	JNK	Shown to increase period in mammalian tissues and cells *in vitro*, also inhibits CK1, *in vitro*	Chansard et al, Neuroscience, 2007; Isojima et al, PNAS, 2009 Zhang et al, Cell, 2009
THFA	adenylyl cyclase	Increases period in mammalian tissues *in vitro* and *in vivo*	O'Neill et al, Science, 2007
9-CPA	adenylyl cyclase	Increases period in mammalian tissues *in vitro*	O'Neill et al, Science, 2007
calyculin A	PP2A	Increases period in mammalian tissues *in vitro*	Eide et al, Mol Cell Biol, 2005;
geldanamycin	hsp90	Increases free-running period in *Drosophila* (w^{1118})	Hung et al, J Biol Rhythms, 2009
MG132	proteasome	Increases period in mammalian tissues *in vitro*	Eide et al, Mol Cell Biol, 2005;
trichostatin A	Histone deacetylase inhibitor	Regulates clock gene expression in mammalian cells and SCN *in vitro*, reversible histone modification also implicated in other organisms.	Naruse et al, Mol Cell, Biol, 2004. Perales and Mas, Plant Cell, 2007.
SD169	P38α MAPK	Implicated as an output mechanism in *Neurospora* (negative control)	Vitalini, PNAS, 2007

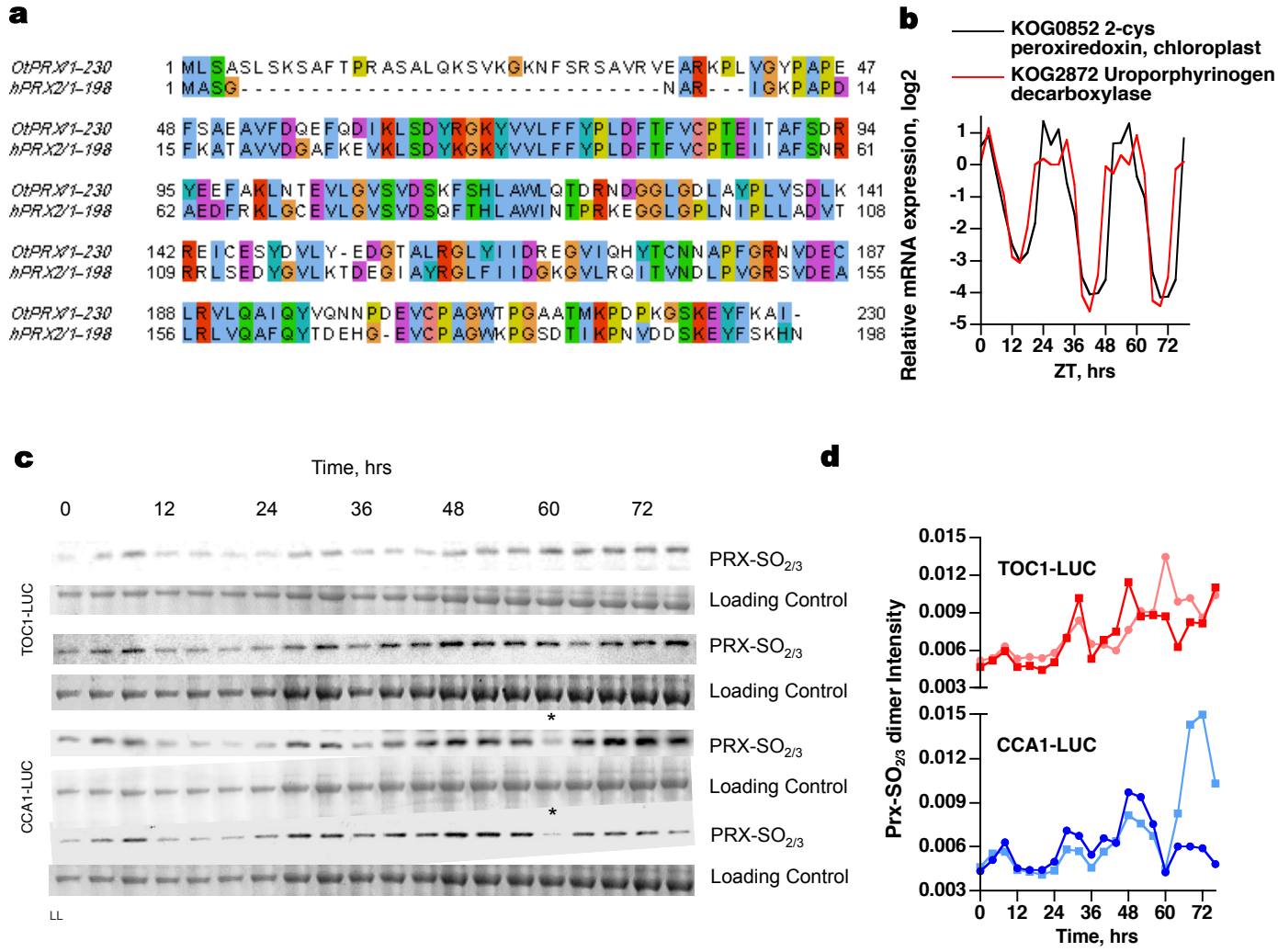
Table S2. A summary of literature reports in other taxa for the reported targets of drugs that modulate free-running period in *Ostreococcus tauri*.

O'Neill *et al*, Supplementary Figure 1



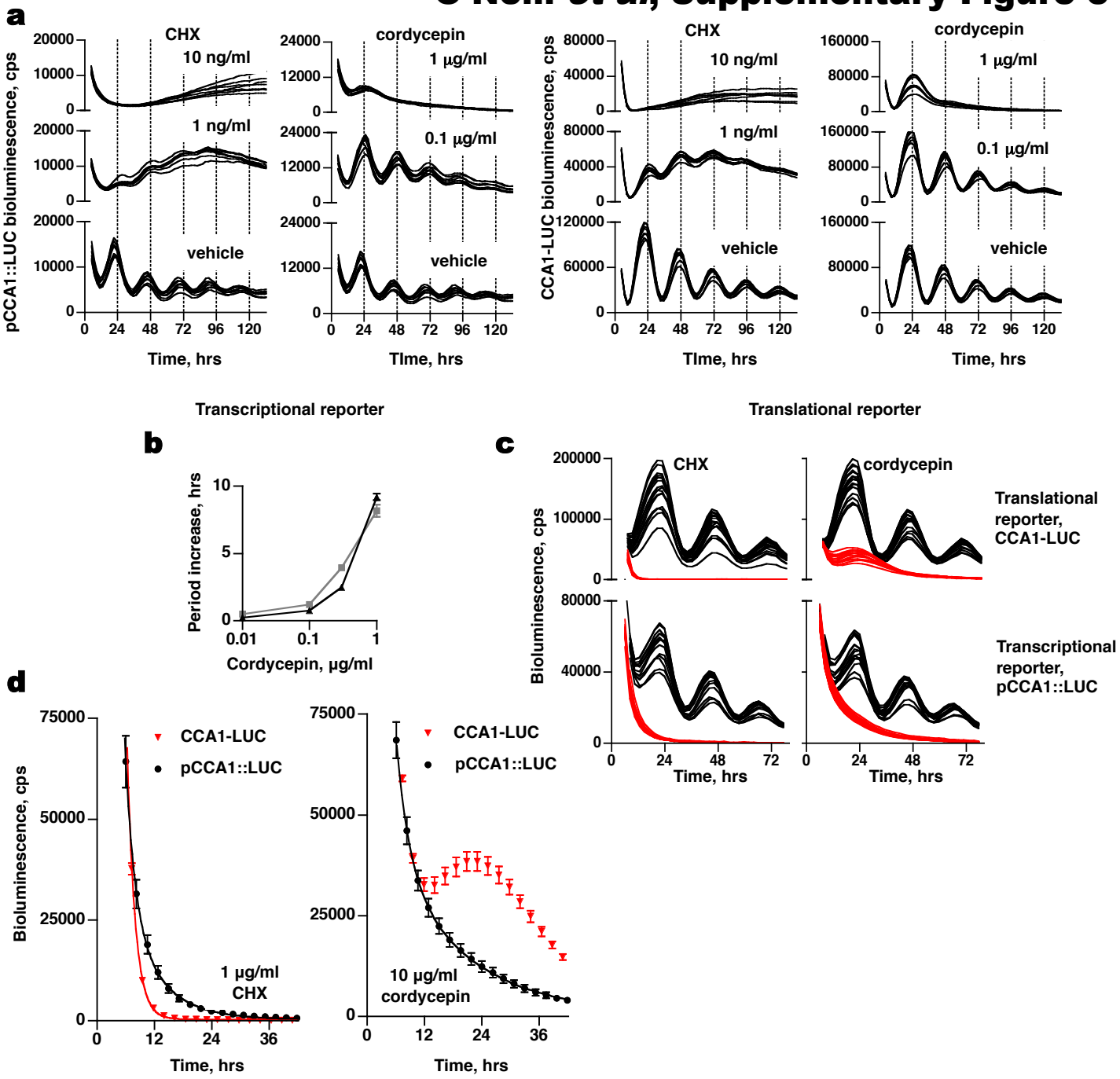
Supplementary Figure 1. Representative plots of circadian rhythms of gene expression in *O. tauri* following constant darkness. Two representative plots showing recovery of rhythmic bioluminescence upon transfer at 4 hour intervals from constant darkness into constant light. An additional 4 hours in constant darkness separate the red from black traces in each case. Whilst plot **a** shows a clear difference in circadian phase, plot **b** does not, a departure from absolute phase resetting by light.

O'Neill *et al*, Supplementary Figure 2



Supplementary Figure 2. Sequence, expression and post-translational modification of PRX in *O. tauri*. **a**, Sequence alignment of *O. tauri* thioredoxin peroxidase and human PRX2, black line indicates the highly conserved region surrounding the catalytic cysteine residue that is pertinent to the following western blots and those in Figure 2. **b**, *O. tauri* PRX is rhythmically transcribed under 12:12 hour light:dark cycles in phase with other afternoon-expressed transcripts (adapted from Monnier *et al*, 2010, BMC Genomics). **c**, Immunoblots show altered rhythms of hyperoxidised peroxiredoxin (PRX-$SO_{2/3}$) in constant light for mutant *O. tauri* lines that express an additional genomic copy of TOC1 (TOC1-LUC), previously shown to display longer period under constant conditions compared with lines expressing an extra copy of CCA1 (CCA1-LUC), indicated by the decrease in signal at 60 hours in the controls (*). **d**, Plots of individual intensities from **c.**

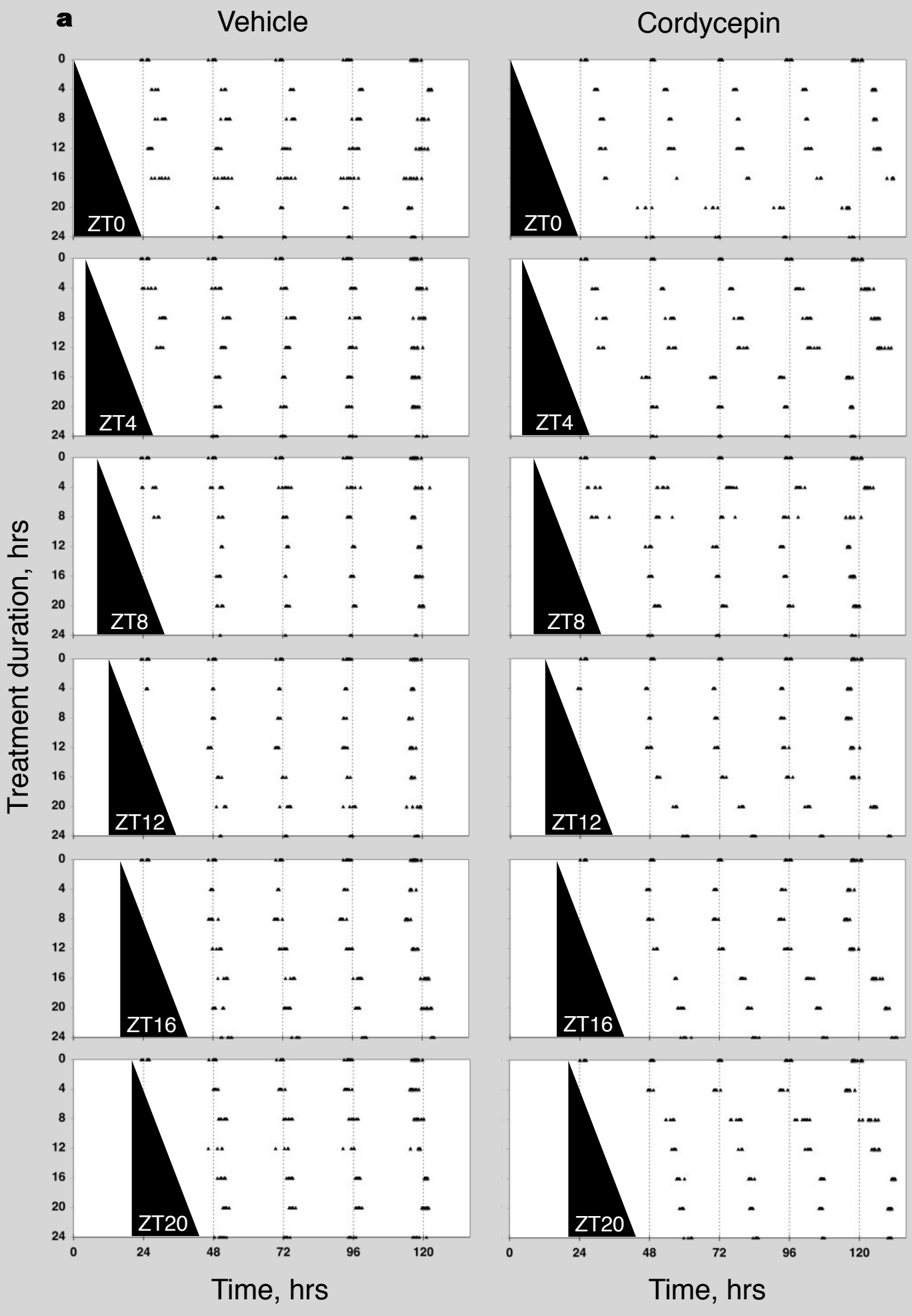
O'Neill *et al*, Supplementary Figure 3



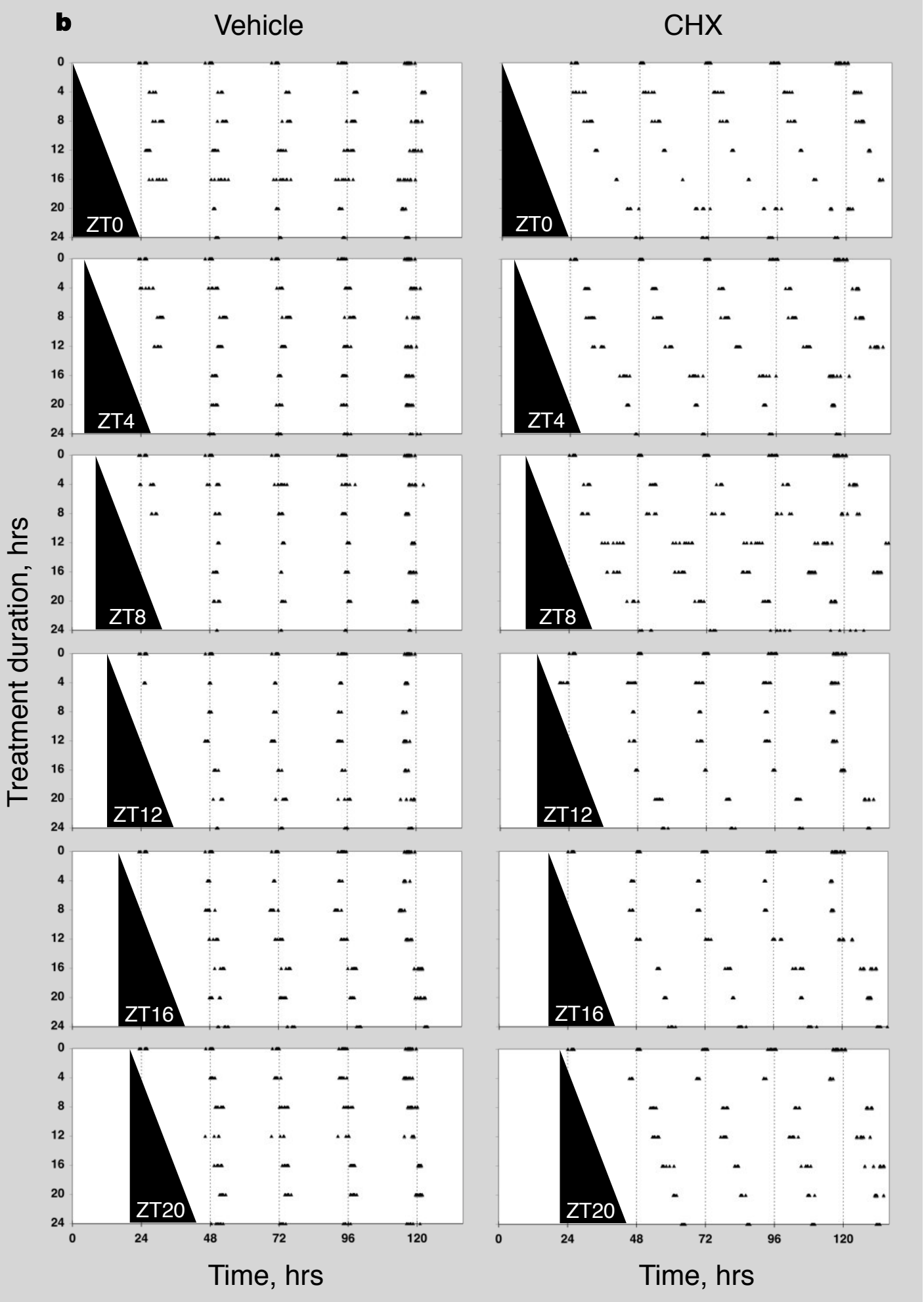
Supplementary Figure 3. The effect of inhibition of cellular RNA synthesis or cytosolic translation upon transcriptional and translational bioluminescent clock reporters under constant light.

a, Representative plots showing the effect of increasing concentrations of CHX and cordycepin on transcriptional and translational reporters (n = 8). **b**, The effect on circadian period due to reduced cellular transcription rates (black, translational reporter; grey, transcriptional reporter; error bars ± SEM; 2-way ANOVA, $p < 0.0001$ for concentration effect, n = 8). **c**, The effect of maximal inhibition of transcription (cordycepin) or cytosolic translation (CHX) upon transcriptional or translational bioluminescent clock reporters (black, vehicle; red, drug treatment; n=16). **d**, Expanded from **c**, grouped data from lines expressing the translational CCA1-LUC reporter in the presence of 10 µg/ml cordycepin deviate from the exponential decay exhibited by the transcriptional pCCA1::LUC reporter under the same conditions, or either reporter in the presence of 1 µg/ml CHX ($R^2 \geq 0.98$ for all three curve fits; error bars ± SEM, n = 16).

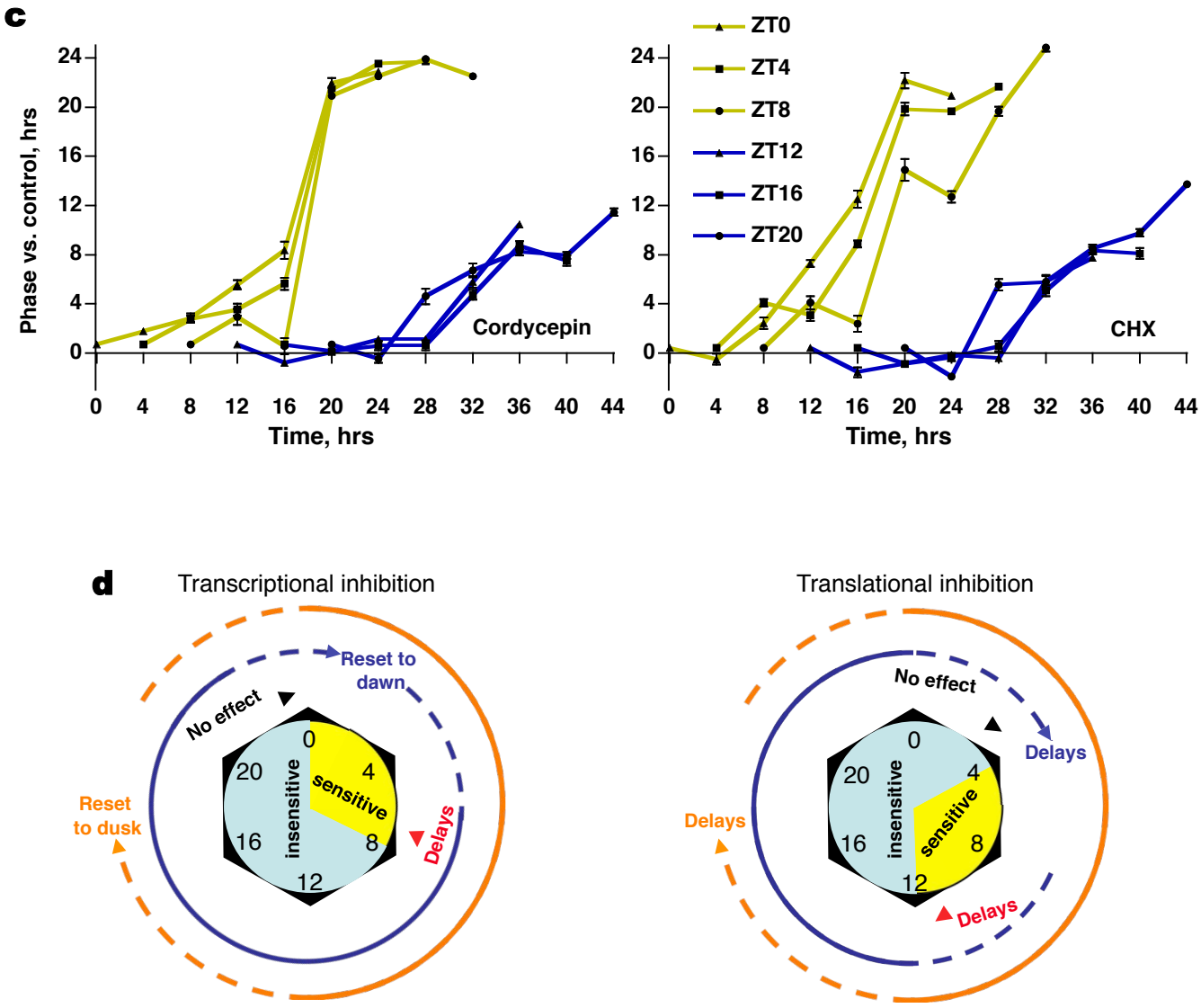
O'Neill *et al*, Supplementary Figure 4-1



O'Neill *et al*, Supplementary Figure 4-2

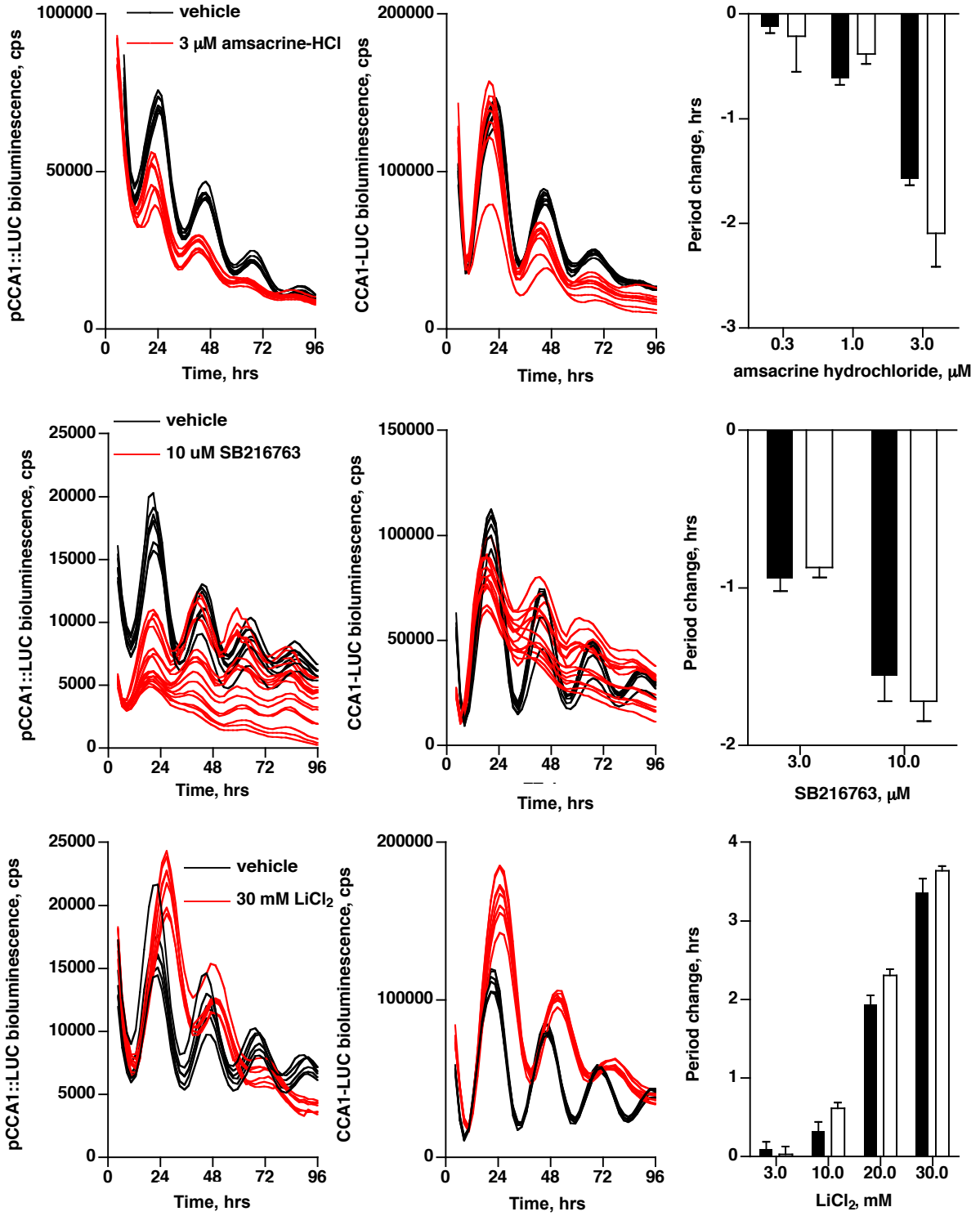


O'Neill *et al*, Supplementary Figure 4-3

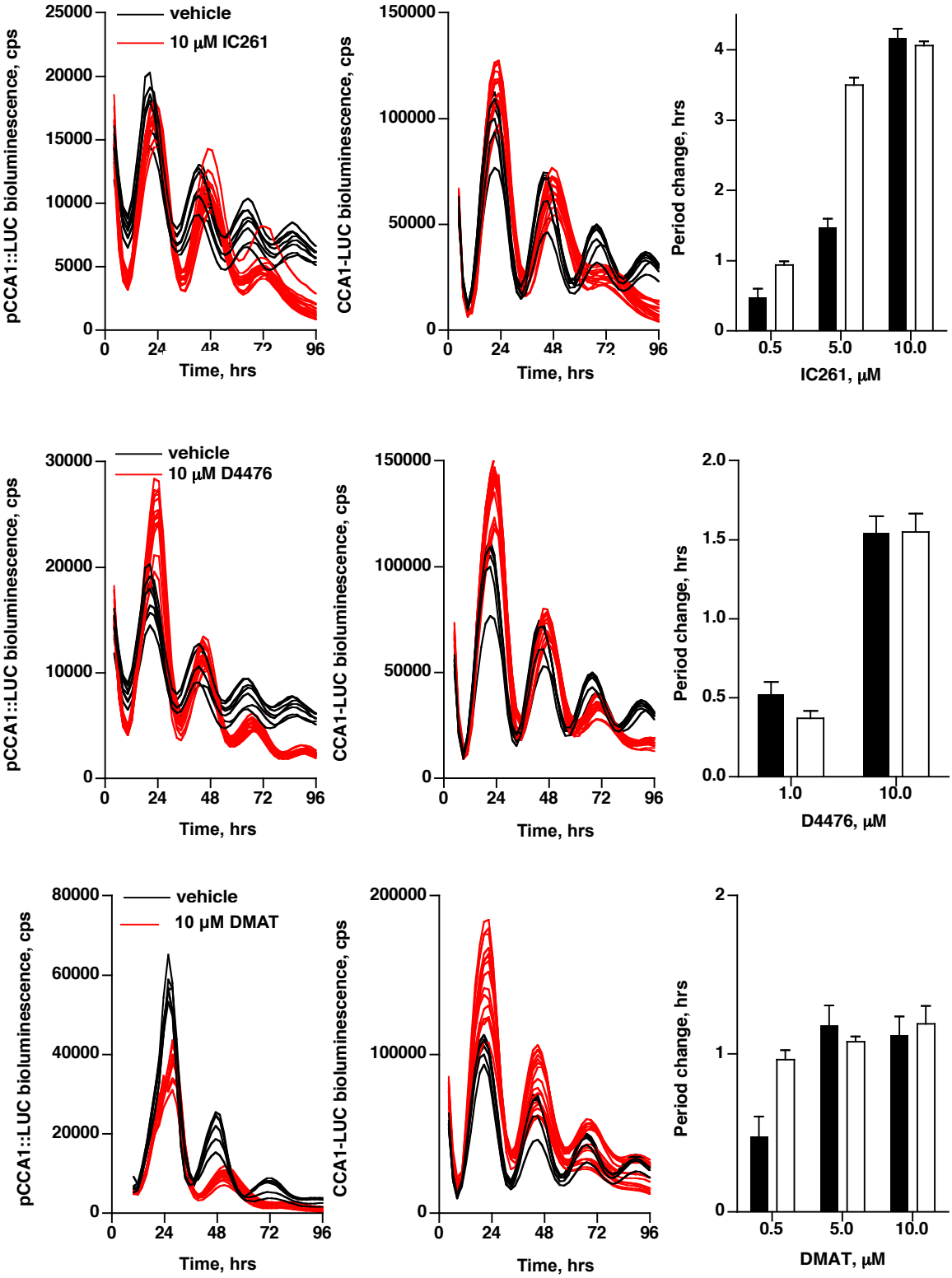


Supplementary Figure 4. Individual and grouped data from chemical 'wedge' experiment. a, Peak times of CCA1-LUC expression in constant light for individual replicates entrained under LD, following treatment with vehicle or cordycepin starting from ZT0, 4, 8, 12, 16, or 20 extending for 4-hour increments up to 24 hours (n>5). **b**, Peak times of CCA1-LUC expression in constant light for individual replicates entrained under LD, following treatment with vehicle or CHX starting from ZT0, 4, 8, 12, 16, or 20 extending for 4-hour increments up to 24 hours (n>5). **c**, An alternate plot of Figure 3c shows experimentally derived phases relative to vehicle controls (n > 5, error bars ± SEM). Yellow and blue lines indicate treatments beginning during subjective day and night, respectively. **d**, Cartoon schematic summarizing the experimental data shown in **a**, **b** and **c**; dotted lines represent different windows for treatment start times, solid lines represent continued treatment, arrows represent end of treatment with text describing the effect on phase relative to controls.

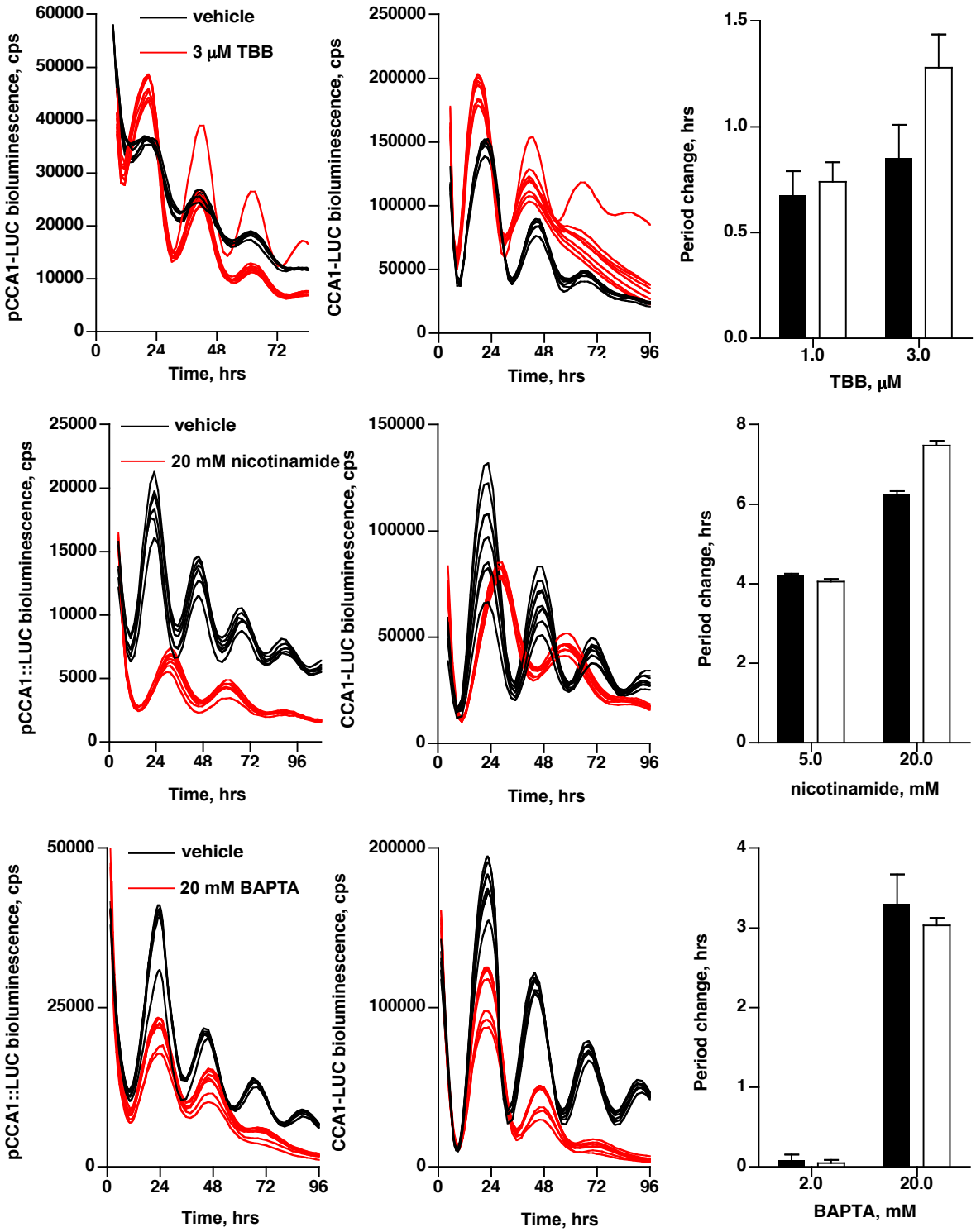
O'Neill *et al*, Supplementary Figure 5-1



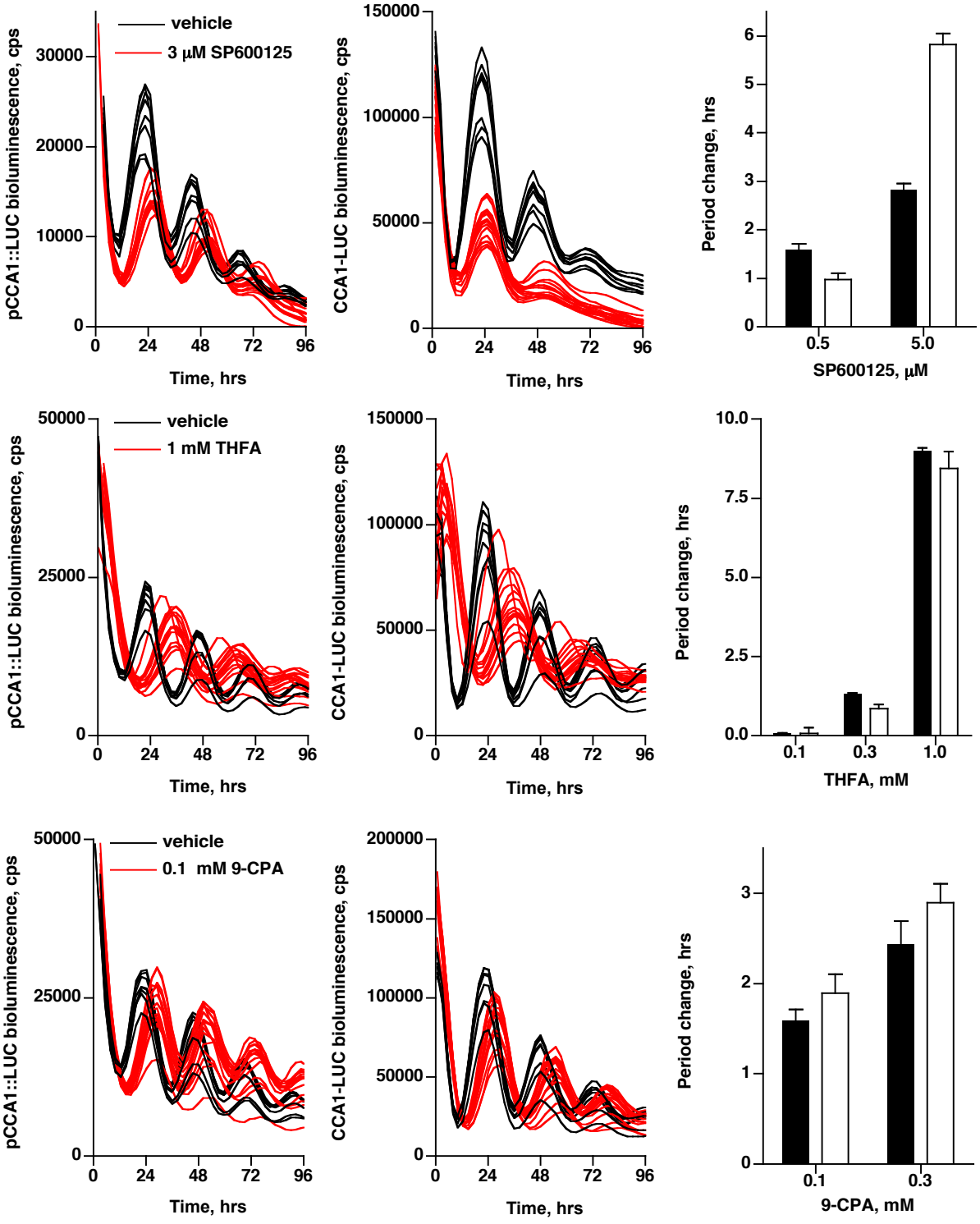
O'Neill *et al*, Supplementary Figure 5-2



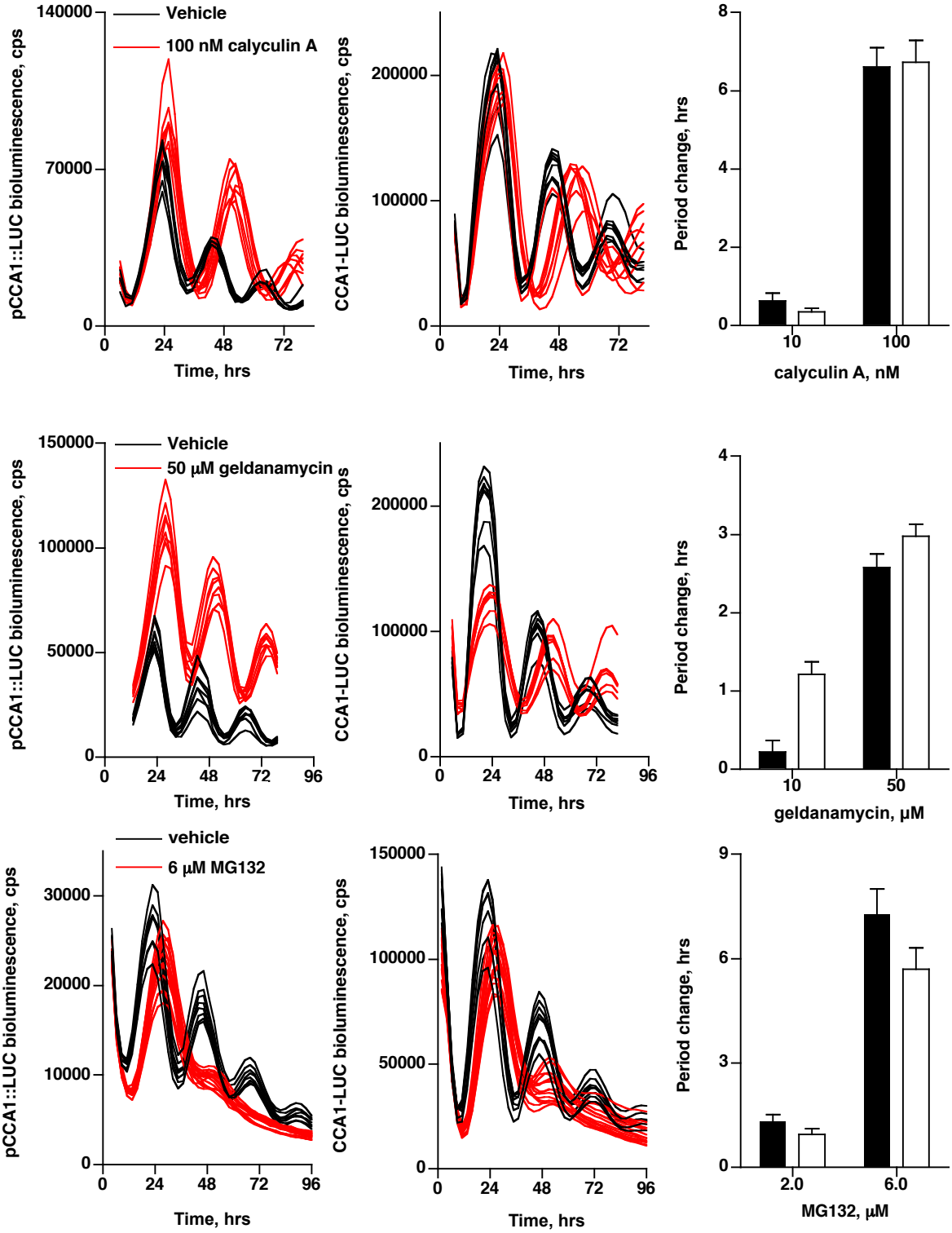
O'Neill *et al*, Supplementary Figure 5-3



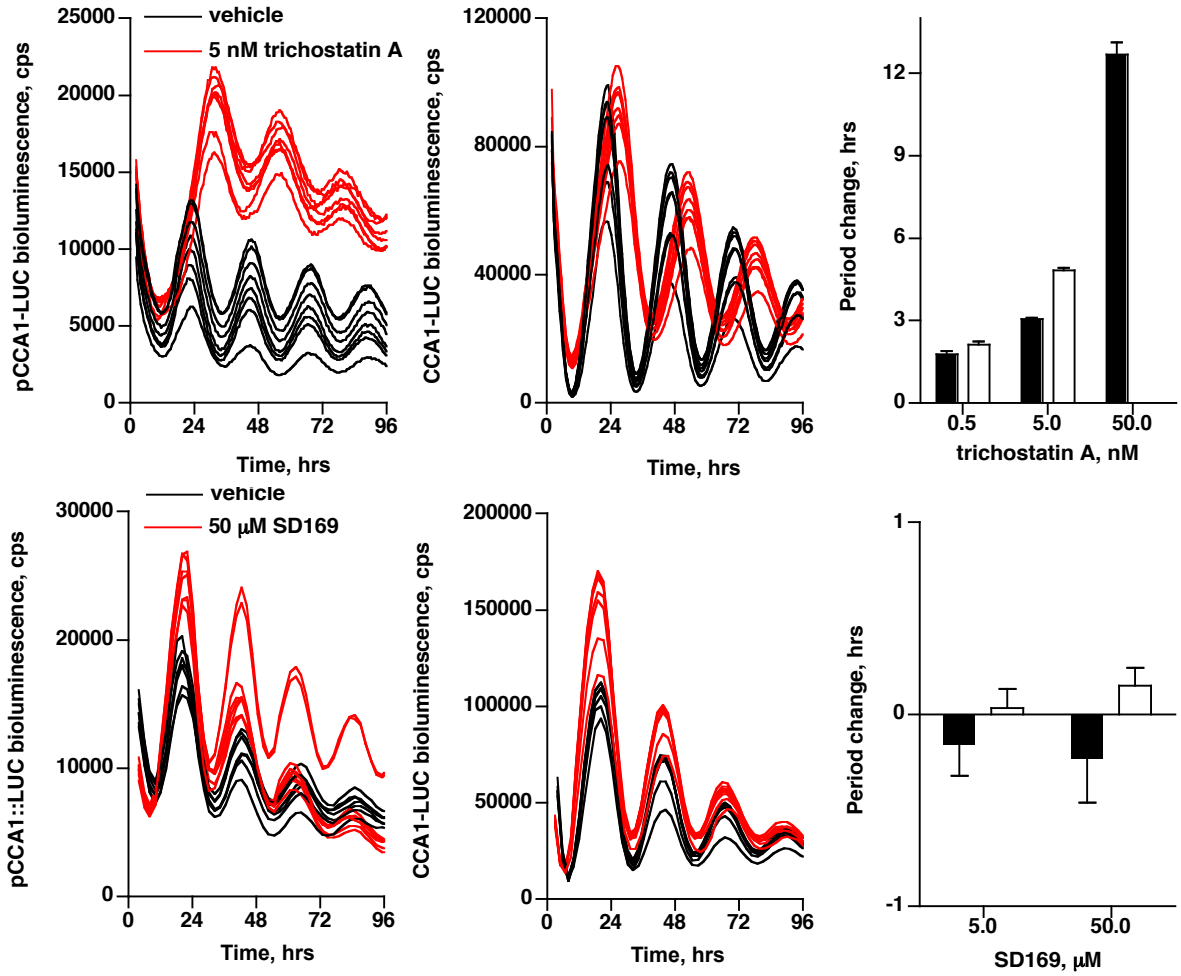
O'Neill *et al*, Supplementary Figure 5-4



O'Neill *et al*, Supplementary Figure 5-5

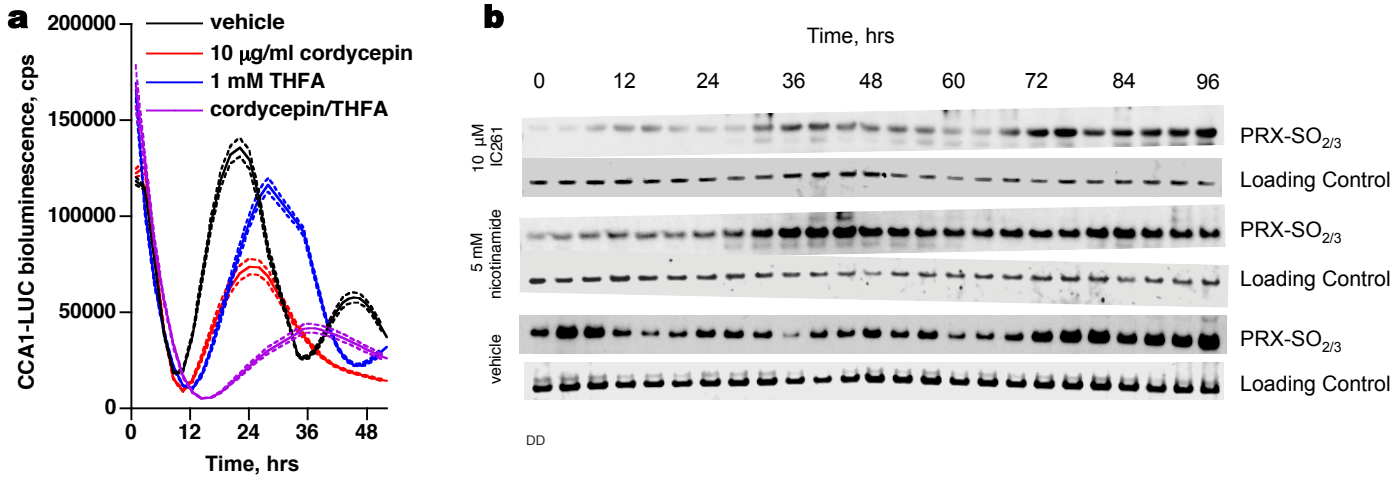


O'Neill *et al*, Supplementary Figure 5-5



Supplementary Figure 5. Representative and grouped data showing similar pharmacological actions on circadian period in *O. tauri* to those reported in other taxa. Representative plots from transcriptional and translational reporter lines with grouped data showing period change relative to vehicle controls (white bars, pCCA1::LUC; black bars, CCA1-LUC; ±SEM, 2-way ANOVA for concentration effect $p < 0.0001$ for all compounds except control compound SD169, $p = 0.90$, $n \geq 8$).

O'Neill *et al*, Supplementary Figure 6



Supplementary Figure 6. Representative and grouped data showing the effects of pharmacological modulators of circadian period in *O. tauri*, in the absence of transcription. a, Grouped data showing CCA1-LUC reporter line under LL in the presence of cordycepin, THFA or both (n=8, mean ±SEM, dotted line). **b**, Immunoblots showing the effect of 5 mM nicotinamide or 10 µM IC261 on rhythms in PRX-$SO_{2/3}$ vs. vehicle in constant darkness.