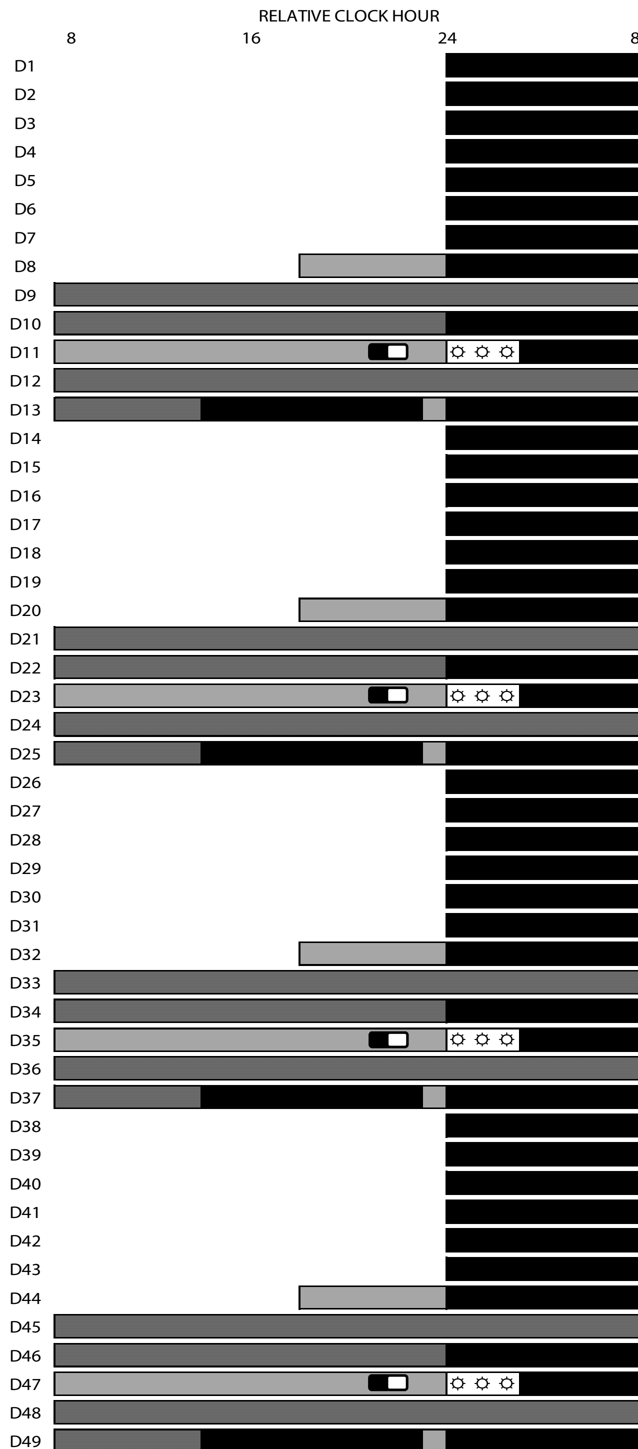
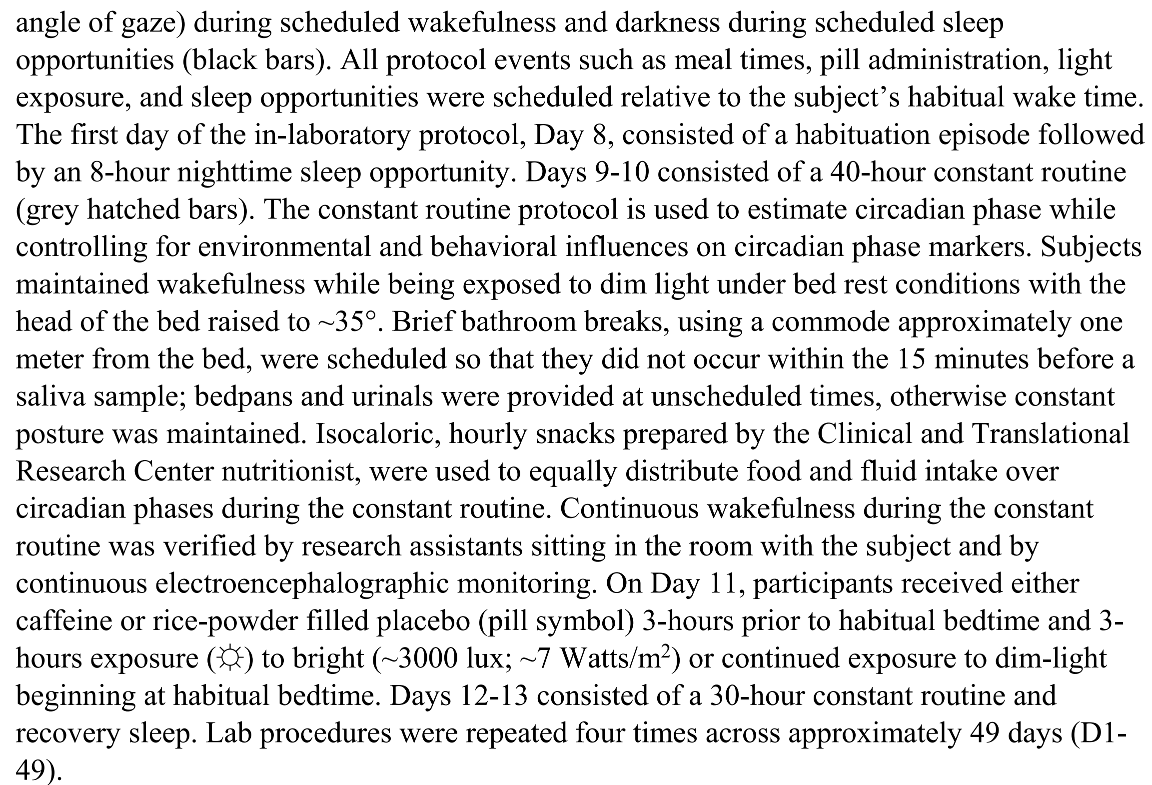


Fig S1. 49 day protocol for human experiment. Each subject participated in an approximate 49 day-long protocol that included exposure to four randomly ordered conditions: dim light-placebo, dim light-caffeine, bright light-placebo, bright light-caffeine. The order of conditions was different for each subject and based on a Latin square design. Following approximately seven days of ambulatory monitoring prior to each laboratory visit, participants lived in an environment free of external time cues under temperature controlled (~22.2 °C), dim light conditions equivalent to candle light (~1.9 lux; ~0.6 Watts/m^2 in the



angle of gaze) during scheduled wakefulness and darkness during scheduled sleep opportunities (black bars). All protocol events such as meal times, pill administration, light exposure, and sleep opportunities were scheduled relative to the subject's habitual wake time. The first day of the in-laboratory protocol, Day 8, consisted of a habituation episode followed by an 8-hour nighttime sleep opportunity. Days 9-10 consisted of a 40-hour constant routine (grey hatched bars). The constant routine protocol is used to estimate circadian phase while controlling for environmental and behavioral influences on circadian phase markers. Subjects maintained wakefulness while being exposed to dim light under bed rest conditions with the head of the bed raised to ~35°. Brief bathroom breaks, using a commode approximately one meter from the bed, were scheduled so that they did not occur within the 15 minutes before a saliva sample; bedpans and urinals were provided at unscheduled times, otherwise constant posture was maintained. Isocaloric, hourly snacks prepared by the Clinical and Translational Research Center nutritionist, were used to equally distribute food and fluid intake over circadian phases during the constant routine. Continuous wakefulness during the constant routine was verified by research assistants sitting in the room with the subject and by continuous electroencephalographic monitoring. On Day 11, participants received either caffeine or rice-powder filled placebo (pill symbol) 3-hours prior to habitual bedtime and 3-hours exposure (☼) to bright (~3000 lux; ~7 Watts/m^2) or continued exposure to dim-light beginning at habitual bedtime. Days 12-13 consisted of a 30-hour constant routine and recovery sleep. Lab procedures were repeated four times across approximately 49 days (D1-49).

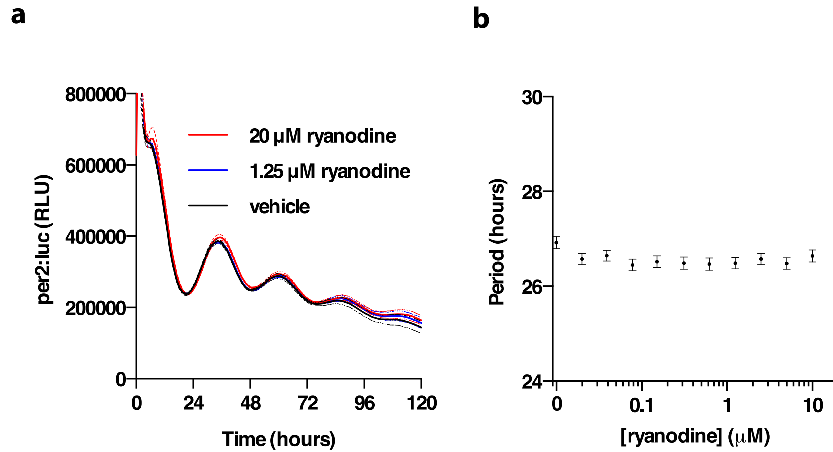
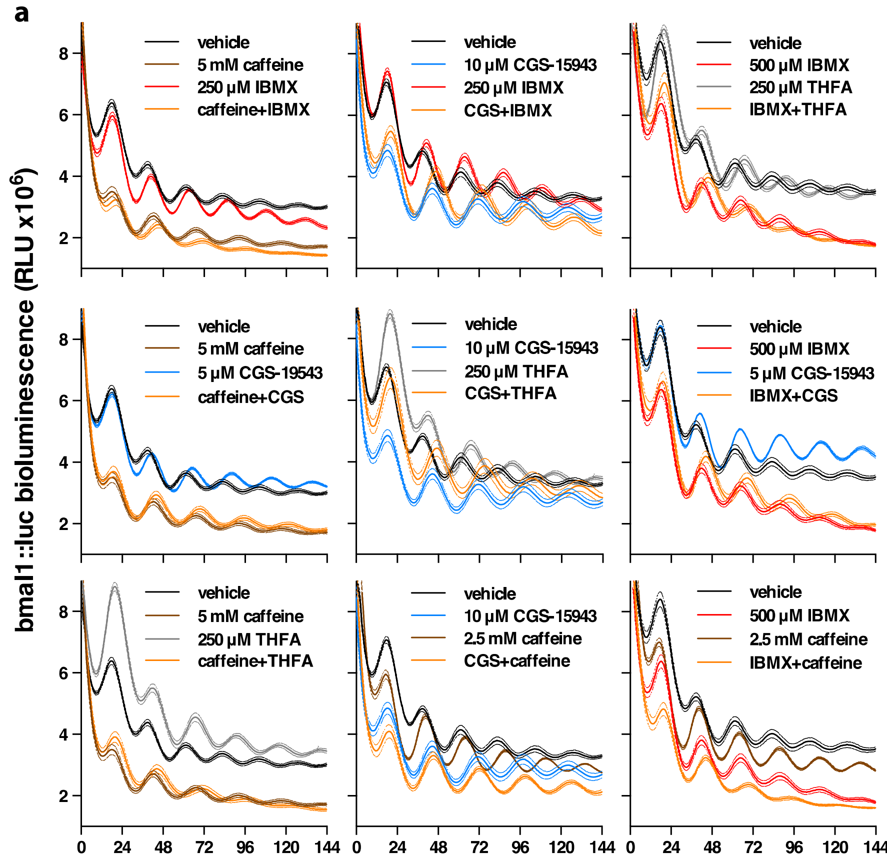
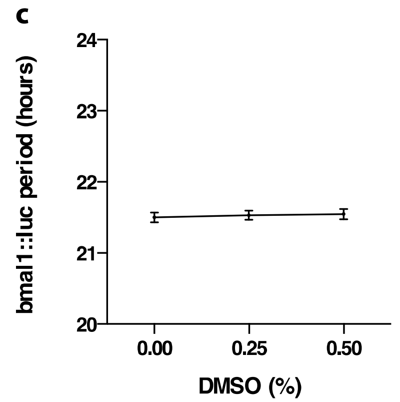
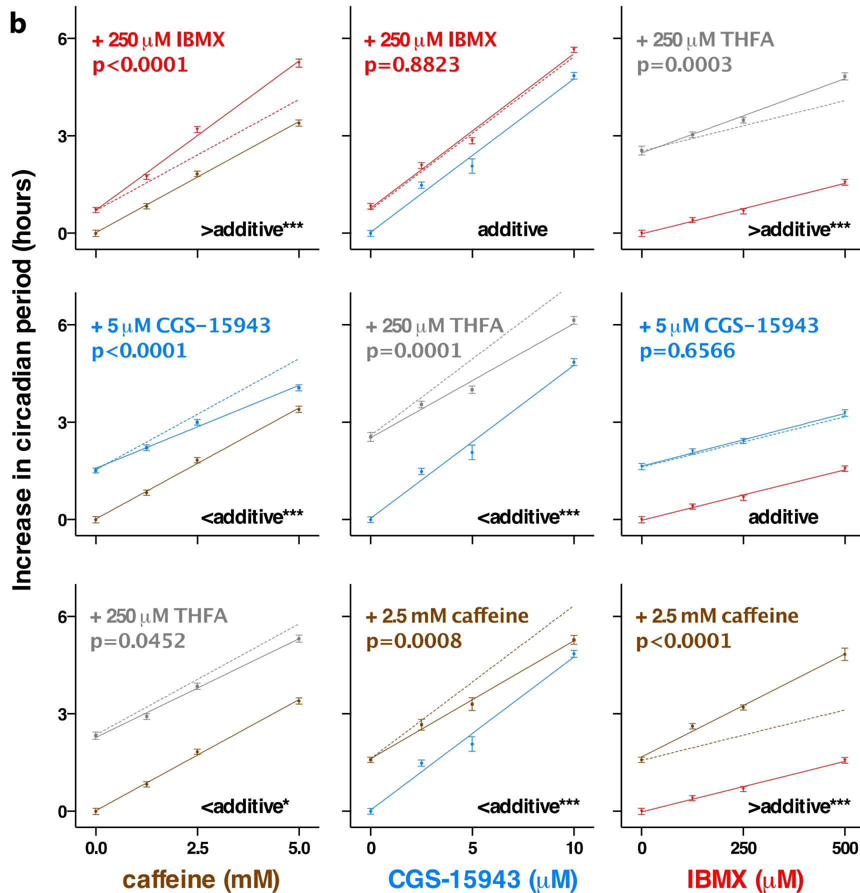


Fig S2. Ryanodine does not lengthen circadian period reported by per2:luc in cultured human cells *in vitro*. (A) Representative grouped bioluminescence data, n=4, mean + SEM. (B) Ryanodine does not lengthen circadian period in U2OS cells at any concentration tested, n=4, mean ± SEM, p=0.11 by one-way ANOVA, p≥0.12 for every drug concentration vs. vehicle control by Bonferroni's multiple comparisons test.

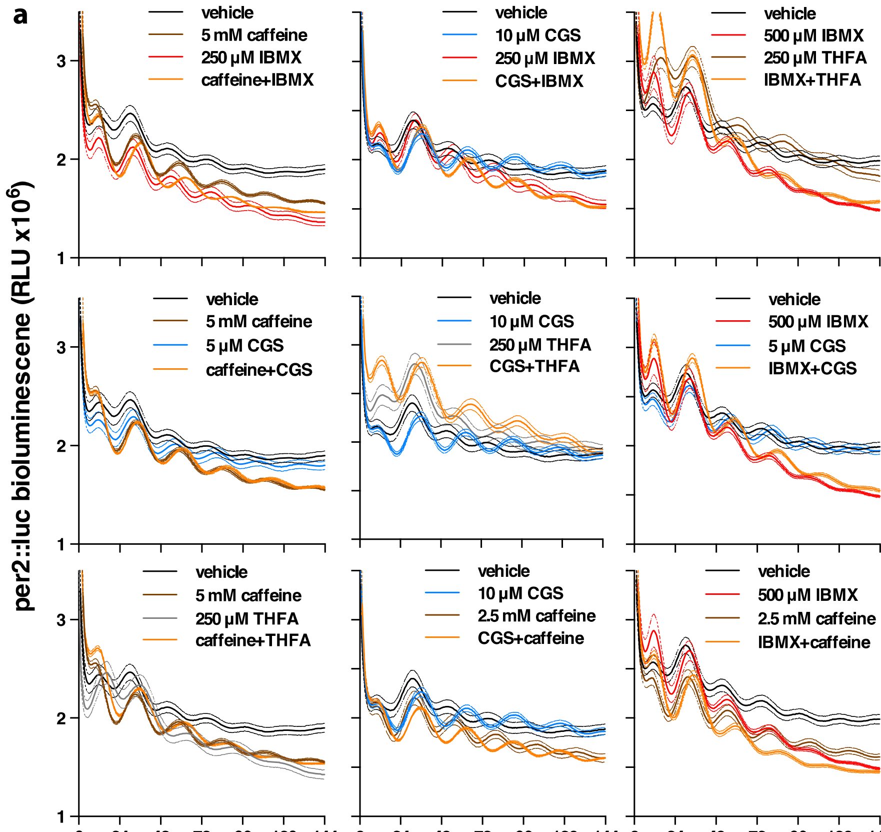


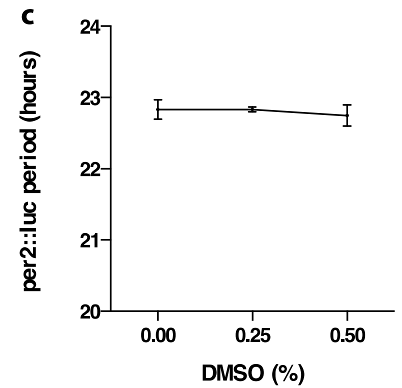


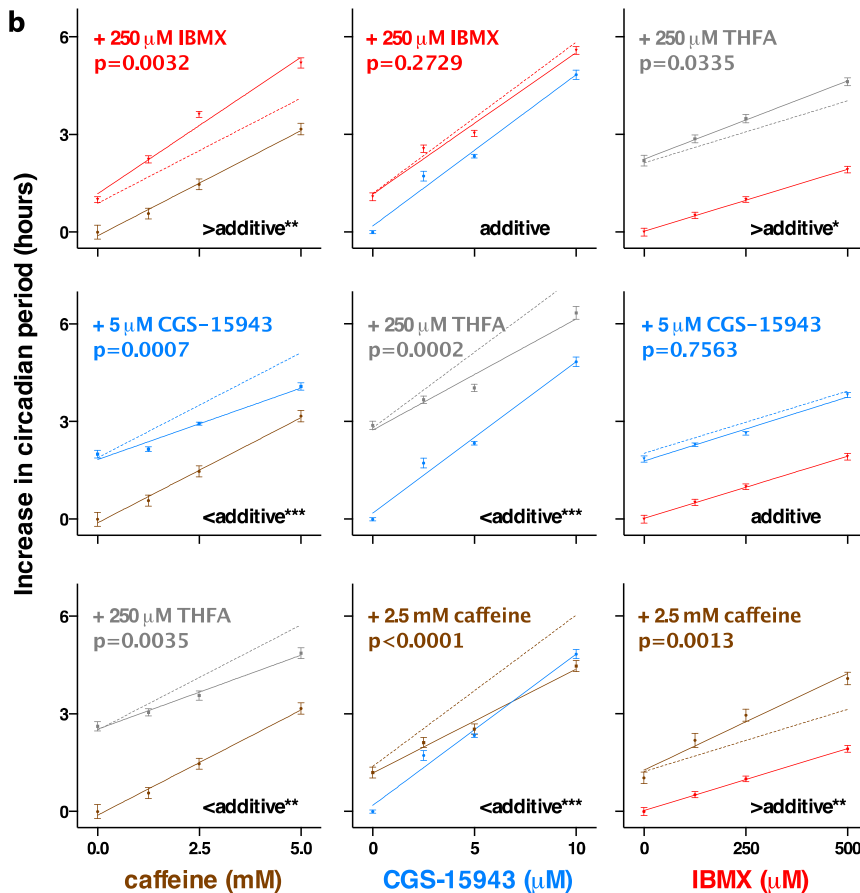


Summary. For each panel:
Data points ± SEM: circadian period of human U2OS cells at each drug concentration (axis labels at bottom).
Lower solid lines: linear regression for dose-dependent period lengthening in response to a single drug applied continuously (**simple dose response**)
Dotted lines: null hypothesis, expected dose-dependent period lengthening in the presence of an additional drug (of fixed concentration) assuming the two drugs act additively and act at different cellular targets (**null hypothesis**)
Upper colored lines: linear regression for actual dose-dependent period lengthening in the presence of an additional drug at fixed concentration, labeled in color on each panel (**observed**)
For the linear regression slope of each panel, if:
observed > **null**, then >additive or synergistic
Conclude different drug targets
observed = **null**, then additive
observed < **null**, then <additive
Conclude common drug targets

Fig S3. Caffeine increases bmal1:luciferase circadian period in cultured human cells *in vitro*, in an adenosine receptor/cAMP-dependent fashion. (A) Representative examples of grouped raw bioluminescence data (mean ± SEM, n=6) showing the effect of different concentrations and combinations of drugs on human U2OS cells stably expressing bmal1:luciferase, a reporter of cellular timekeeping. (B) Grouped data points (mean period ± SEM, n=6) showing dose-dependent lengthening of circadian period in response to caffeine (left 3 panels), CGS-15943 (central 3 panels), and IBMX (right 3 panels) ± a fixed concentration of a second period-lengthening drug (250 μM IBMX, red; 5 μM CGS-15943, blue; 2.5 mM caffeine, brown; 250 μM THFA, grey). The single-drug dose-response begins at the origin in each case, with solid lines being the linear regression for each condition ($R2 \geq 0.98$). Broken colored lines represent the null hypothesis (simple additive drug action i.e. a y-transformation with no change in the slope of the dose response). At the top left of each sub-panel, sum-of-squares F-test p-values are reported, where $p<0.05$ indicates rejection of the null hypothesis (same slope for both groups). The significance and drug additivity are summarized at the bottom right of each sub-panel. (C) DMSO concentration does not affect cellular timekeeping, $p=0.88$ by 1-way ANOVA, n=6.







Summary. For each panel:

Data points ± SEM: circadian period of human U2OS cells at each drug concentration (axis labels at bottom).

Lower solid lines: linear regression for dose-dependent period lengthening in response to a single drug applied continuously (**simple dose response**)

Dotted lines: null hypothesis, expected dose-dependent period lengthening in the presence of an additional drug (of fixed concentration) assuming the two drugs act additively and act at different cellular targets (**null hypothesis**)

Upper colored lines: linear regression for actual dose-dependent period lengthening in the presence of an additional drug at fixed concentration, labeled in color on each panel (**observed**)

For the linear regression slope of each panel, if:

observed > **null**, then >additive or synergistic
Conclude different drug targets

observed = **null**, then additive

observed < **null**, then <additive
Conclude common drug targets

Fig S4. Caffeine increases circadian period reported by per2:luc in cultured human cells *in vitro*, in an adenosine receptor/cAMP-dependent fashion.

(A) Representative examples of grouped raw bioluminescence data (mean ± SEM, n=6) showing the effect of different concentrations and combinations of drugs on human U2OS cells stably expressing bmal1:luciferase, a reporter of cellular timekeeping. (B) Grouped data points (mean period ± SEM, n=6) showing dose-dependent lengthening of circadian period in response to caffeine (left 3 panels), CGS-15943 (central 3 panels), and IBMX (right 3 panels) ± a fixed concentration of a second period-lengthening drug (250 µM IBMX, red; 5 µM CGS-15943, blue; 2.5 mM caffeine, brown; 0.25 mM THFA, grey). The single-drug dose-response begins at the origin in each case, with solid lines being the linear regression for each condition ($R^2 \geq 0.98$). Broken colored lines represent the null hypothesis (simple additive drug action i.e. a y-transformation with no change in the slope of the dose response). At the top left of each sub-panel, sum-of-squares F-test p-values are reported, where $p<0.05$ indicates rejection of the null hypothesis (same slope for both groups). The significance and drug additivity are summarized at the bottom right of the sub-panel. (C) DMSO concentration does not affect cellular timekeeping, p=0.88 by 1-way ANOVA, n=6.

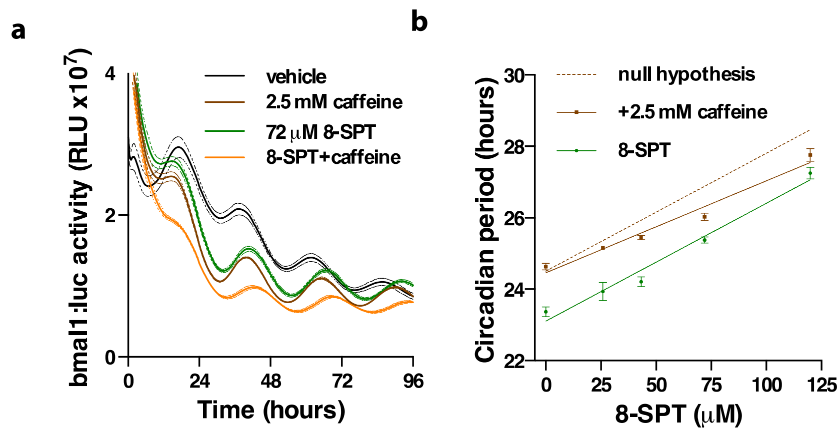
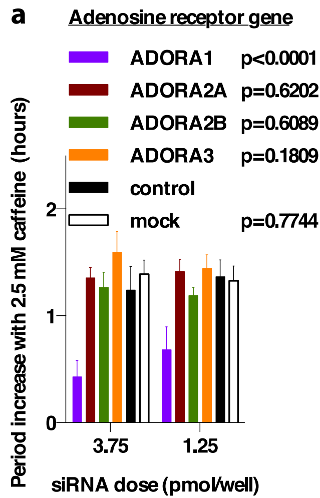
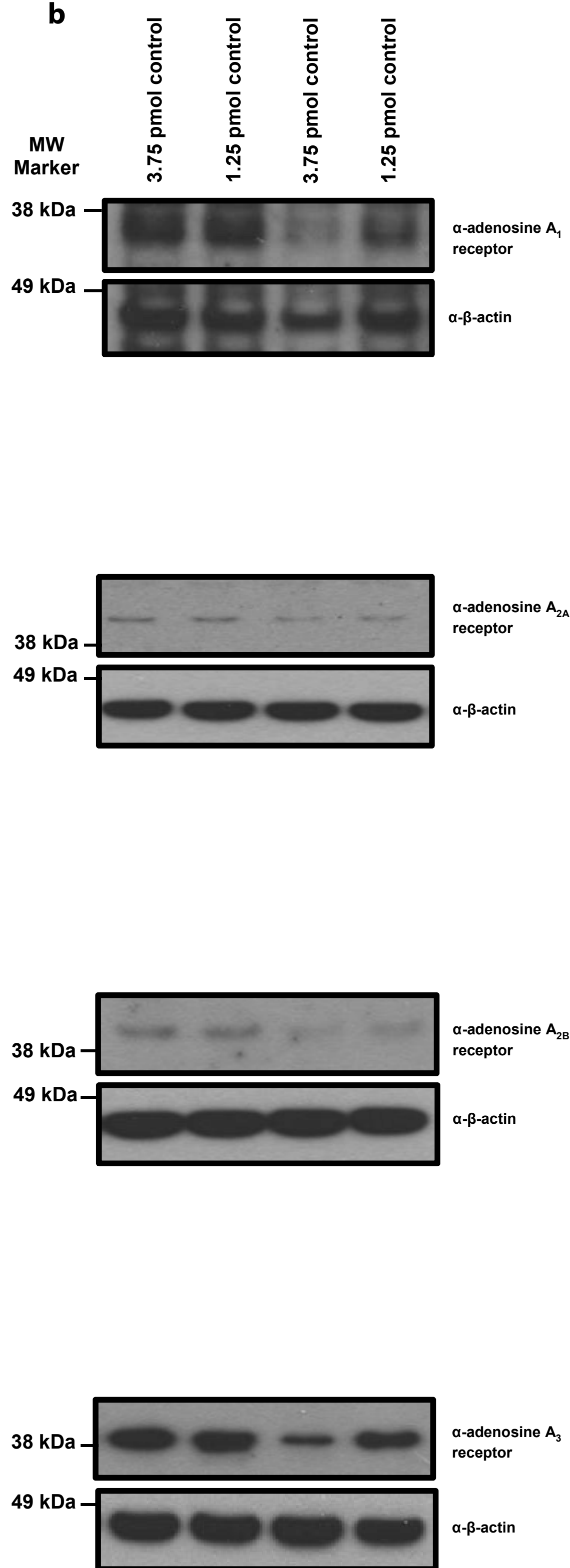


Fig S5. Caffeine acts at the same site as 8-(p-sulfophenyl)theophylline (8-SPT) to increase circadian period in cultured human cells *in vitro*.

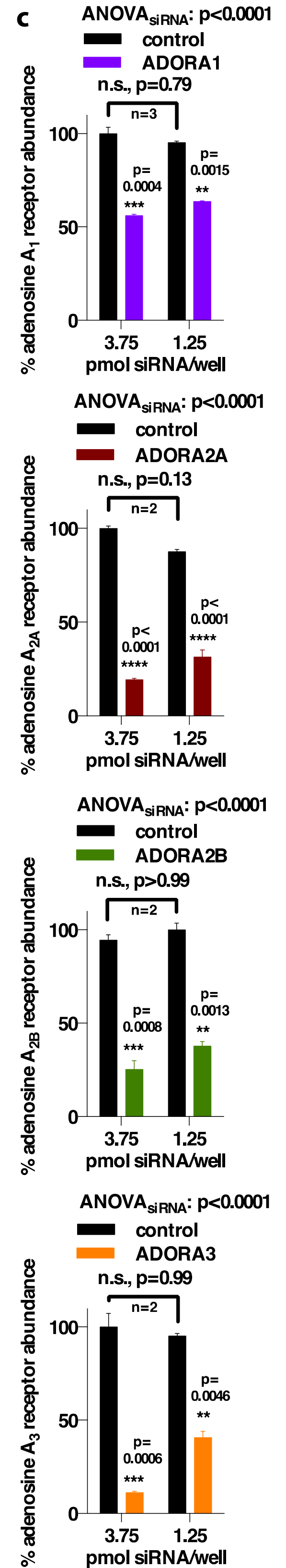
(A) Representative examples of grouped raw bioluminescence data (mean ± SEM, n=4) show period lengthening due to 8-SPT ± caffeine. (B) Grouped data points (period mean ± SEM, n=4) showing that the additional presence of 2.5 mM caffeine (brown) significantly reduces the slope of dose-dependent period lengthening elicited by 8-SPT (green). The sum-of-squares F-test p-value is reported, where $p<0.05$ indicates rejection of the null hypothesis (same slope for both groups). Solid lines, linear regression ($R^2 \geq 0.94$). Broken brown line represents the null hypothesis. Red arrow indicates that combined drug effect is significantly less than additive.



a
Adenosine receptor gene
ADORA1 p<0.0001
ADORA2A p=0.6202
ADORA2B p=0.6089
ADORA3 p=0.1809
control
mock p=0.7744
Period increase with 2.5 mM caffeine (hours)
siRNA dose (pmol/well)



b
MW Marker
3.75 pmol control
1.25 pmol control
3.75 pmol control
1.25 pmol control
38 kDa
49 kDa
α-adenosine A1 receptor
α-β-actin
α-adenosine A2A receptor
α-adenosine A2B receptor
α-adenosine A3 receptor



c
ANOVAsiRNA: p<0.0001
control
ADORA1
n.s., p=0.79
n=3
p= 0.0004 ***
p= 0.0015 **
% adenosine A1 receptor abundance
pmol siRNA/well
ADORA2A
n.s., p=0.13
n=2
p< 0.0001 ****
% adenosine A2A receptor abundance
ADORA2B
n.s., p>0.99
p= 0.0008 ***
p= 0.0013 **
% adenosine A2B receptor abundance
ADORA3
n.s., p=0.99
p= 0.0006 ***
p= 0.0046 **
% adenosine A3 receptor abundance

Fig S6. Quantification of adenosine receptor siRNA knockdown efficacy

(A) siRNA targeting ADORA1 significantly attenuates the period lengthening action of caffeine in U2OS cells ($n \geq 5$), mean ± SEM. By two-way ANOVA, $p<0.0001$ for siRNA effect. Uncorrected Fisher's LSD p-values for each siRNA vs. control are reported to the right of each gene transcript targeted. (B) Representative western blots of U2OS cells transfected with siRNA against each of the four human adenosine receptors (A_1, A_{2A}, A_{2B}, A_3) compared with control siRNA sequence (sampled at 72 hours after transfection). (C) Grouped quantification of adenosine receptor protein abundances, normalized to β-actin intensity, mean ± SEM. Replicate numbers ($n \geq 2$ per condition) are reported in each sub-panel, as are 2-way ANOVA p-values for siRNA effect. Bonferroni's multiple comparisons test p-value for each siRNA concentration, compared with respective control, are also reported in each sub-panel and indicated with asterisks (n.s., not significant)..