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PRESS RELEASE

Release Title: Melanopsin DNA aptamers can regulate input signals of mammalian circadian rhythms by altering the phase of the molecular clock
Release Subtitle: DNA aptamers Melapts to wake up refreshed in the morning.

Overview:
DNA aptamers of melanopsin that regulate the clock hands of biological rhythms were developed by the Toyohashi University of Technology and the National Institute of Advanced Industrial Science and Technology (AIST) group.

DNA aptamers can specifically bind to biomolecules to modify their function, potentially making them ideal oligonucleotide therapeutics. We screened the DNA aptamer melanopsin (OPN4), a blue light photopigment in the retina that plays a key role in the use of light signals to reset the phase of circadian rhythms in the central clock.
First, 15 DNA aptamers of melanopsin (Melapts) were identified following eight rounds of Cell-SELEX using cells expressing melanopsin on the cell membrane. Subsequent functional analysis of each Melapt was performed in a fibroblast cell line stably expressing both *Period2*:ELuc and melanopsin by determining the degree to which they reset the phase of mammalian circadian rhythms in response to blue light stimulation. *Period2* rhythmic expression over a 24-h period was monitored in *Period2:ELuc:* thymidine kinase (TK)*:OPN4* stable fibroblasts expressing melanopsin. At subjective dawn, four Melapts were observed to advance their phase by >1.5 h, whereas seven Melapts delayed their phase by >2 h. A few Melapts caused a phase shift of approximately 2 h, even in the absence of photostimulation, presumably because Melapts can only partially affect input signaling for the phase shift. Additionally, a few Melaps-induced phase shifts in *Period1*::luc transgenic (Tg) mice were used to monitor circadian rhythms by *Period1* rhythmic expression.
These DNA aptamers may have the capacity to affect melanopsin *in vivo*. In summary, Melapts aptamers can successfully regulate the input signal and the shifting phase (both phase advance and phase delay) of mammalian circadian rhythms *in vitro* and *in vivo*.

Details:
Indirectly improving the sleep–wake cycle by manipulating the ability of melanopsin to input signals to the central clock would be socially and economically advantageous.
Melanopsin is a photoreceptor protein expressed in retinal ganglion cells that absorbs blue light with a maximum absorbance of 477 nm. Melanopsin is known to play an important role in resetting the phase of the mammalian circadian clock by blue light and the rhythmic expression of clock genes, such as *Period1,2*

(*Per1,2*). The phase of the molecular circadian clock is reset by and depends on the timing of light stimulation and the transient induction of *Per1* by the melanopsin photoreceptor (Fig. 1). Recently, antagonists of melanopsin acquired via chemical screening of chemical libraries primarily contribute to delaying the rhythm phase.

In this study, we used the cellular systematic evolution of ligands by the exponential enrichment (Cell-SELEX) method to identify DNA aptamers (single-stranded DNA; ssDNA) that cause melanopsin to shift the phase of circadian rhythms. In total, 15 types of melanopsin aptamers (Melapts 1–15) were analyzed to assess their ability to shift the phase of *Per2*::ELuc bioluminescent oscillations in *Per2*:ELuc:TK:Mel stable cells, in which a bioluminescent reporter follows the *Per2* promoter region controlling an enhanced green-emitting luciferase from *Pyrearinus termitilluminans*, with melanopsin overexpressed under the control of the thymidine kinase (TK) promotor. In these stable fibroblast cell lines, the signaling pathway is incorporated into a fibroblast cell that mimics the signaling pathway from the retina to the central master clock (suprachiasmatic nucleus or nuclei: SCN) by melanopsin (Fig. 2).

DNA aptamers are short, single-stranded RNA/DNA molecules that can bind selectively to specific targets, proteins, peptides, and other molecules and can be used clinically to switch the function of target molecules. The main advantages of these aptamers include their high target specificity, lack of immunogenicity, and ease of synthesis.

Among the 15 DNA aptamers of melanopsin (Melapts), four Melapts induced a phase advance and seven Melapts induced a delay in circadian rhythms (by >1.5 h and > 2 h, respectively) in the *Per2*::ELuc cell line. A few Melapts induced phase shifts of approximately 2 h, even in the absence of photostimulation *in vitro*.

Melapt04 and Melapt10 induced a phase advance or delay of the circadian clock by approximately 3 h, respectively, at both CT22 and CT8 during the photo signal input process. This suggests that Melapt04 regulates the phase of circadian rhythms and facilitates falling asleep and waking, mainly via phase advance (Fig. 3–5). Two Melaptes exist that advance and delay the phase shift in the same direction, regardless of the timing of the photostimulus. However, the three Melaptes advanced and delayed the phase shift in opposite directions at dawn and dusk. Therefore, these Melapts are expected to be useful for regulating the phases of rhythms (Fig. 6,7).

We performed *in vivo* experiments similar to the *in vitro* experiments to investigate whether Melapt binding to melanopsin in the retina projecting to the SCN affected the phase shifts of the central clock in the SCN. *Per1*::*luc* transgenic mice: mice in which the *Per1*::*luc* recombinant gene was inserted into the genome of all cells. *Per1*::*luc* is a recombinant gene in which the *Per1* promoter region is followed by a

luciferase enzyme derived from fireflies as a reporter to monitor circadian rhythms.

Eight types of Melapt-causing phase-shift responses in *Per2* expression rhythms in the in vitro experiments were injected into the bulbs of the eyes of *Per1*::luc Tg mice at CT22 (Fig. 8, 9). Melapt01, Melapt03, Melapt04, Melapt07, Melapt09, and Melapt10 displayed phase-shift abilities similar to those of *Per2*:ELuc:TK:Mel stable cells: *in vivo* and *in vitro*.
The effect of Melapt on phase shift in *in vivo* experiments can be predicted from *in vitro* experiments. In addition, the total phase shifts were limited to 3 h in intact animals, regardless of the extent of advance or delay by Melapts in *Per2*:Eluk:TK:Mel cells.

In conclusion

In summary, Melapts were able to regulate input signals and phase shifts to achieve both phase advance and phase delay in mammalian circadian rhythms *in vitro* and *in vivo*.
Melapts could contribute to future research focused on resetting circadian clock phases. Melapts could help us better adapt to modern social life cycles, allow crops and domestic animals to be improved for greater productivity, and help shift workers overcome social jet lag by adjusting the phases of the circadian clock. These Melapts could contribute to resetting the phase of the circadian clocks in photic input pathways.

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Reference:

"Melanopsin DNA aptamers can regulate input signals of mammalian circadian rhythms by altering the phase of the molecular clock**"**
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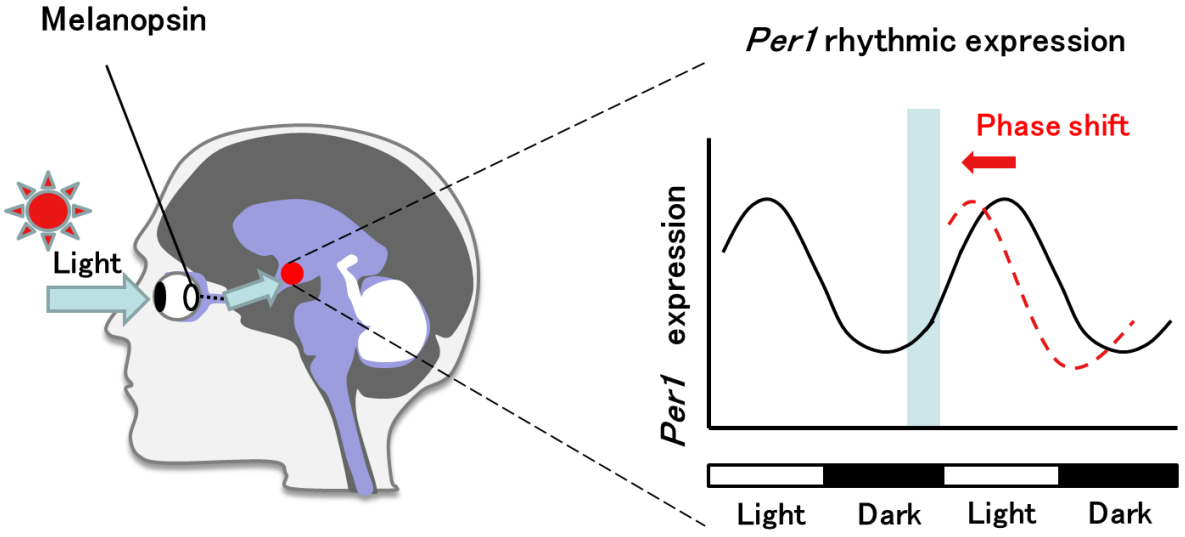

Fig. 1 Phase Shift of Circadian Rhythms by Melanopsin
Melanopsin, a blue photoreceptor in retinal cells, receives blue light each morning and transmits the signal via the nerves of the eye to the SCN, the circadian rhythm central pacemaker. The photo stimulation resets the phase of the circadian rhythms every morning and synchronizes one's own rhythm to the earth's light-dark cycle.

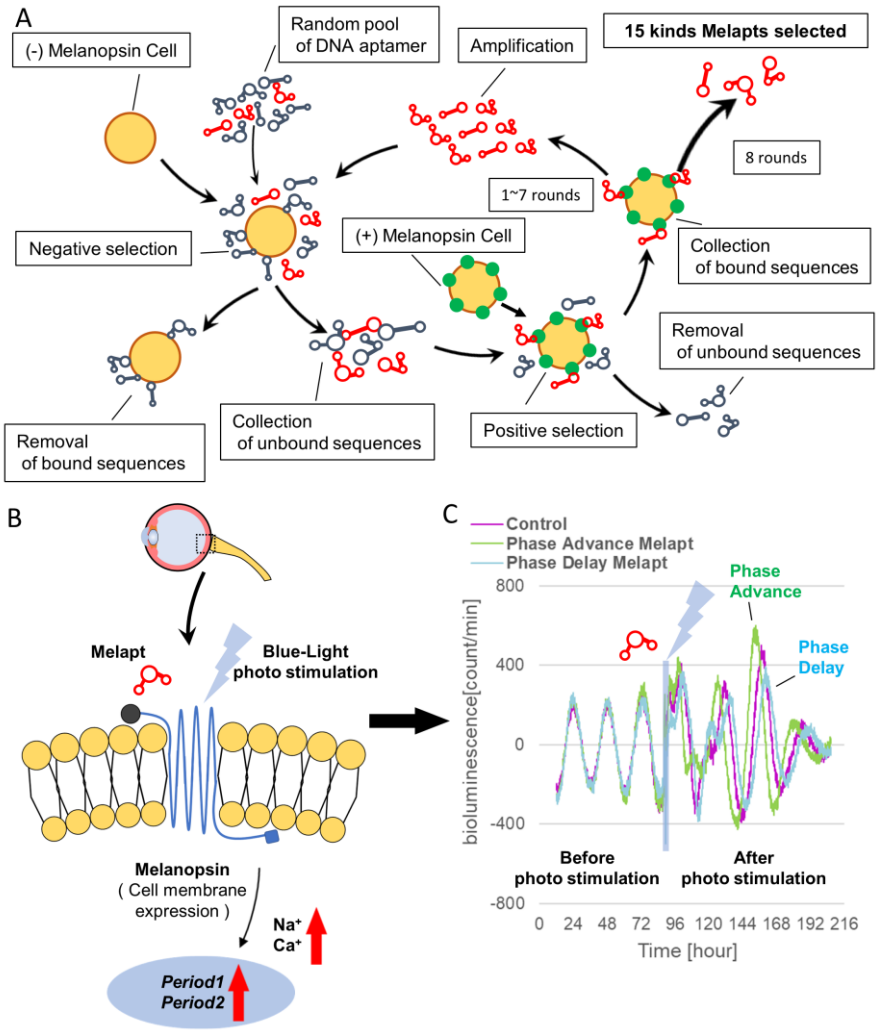

Fig. 2

Cell-SELEX method for screening Melapts. Melapts that specifically bind melanopsin were selected by Cell-SELEX because melanopsin is a membrane protein bound to and accessible from outside cells

(A) DNA aptamers were selected using the Cell-SELEX method from a random ssDNA library. DNA aptamers were mixed and bound to (−) melanopsin cells (negative selection). When unbound DNA aptamers were retrieved, they were combined with (+) melanopsin cells to promote binding to melanopsin protein (positive selection). The Cell-SELEX process was performed over eight rounds, and 15 types of Melapt were screened. (B) Conceptual diagram of Melapt binding to melanopsin followed by induction of the expression of the clock gene *Per2*. When melanopsin expressed on the surface of the cell membrane is photostimulated by a Melapt, the concentration of Na+ and Ca+ in the cytoplasm increases transiently, inducing the activation of transcriptional factors and the upregulation of *Per2* in the cell nucleus. (C) Phase shift of luciferase emission rhythms in *Per2*:ELuc:TK:Mel cells stably expressing melanopsin. When *Per2*:ELuc cells expressing melanopsin were photostimulated post-addition of Melapts, *Per2*:ELuc emission rhythms showed phase advance (green curve) or phase delay (blue curve) relative to controls (purple curve); these shifts were Melapt-dependent. PBS buffer alone was added to the control cells.

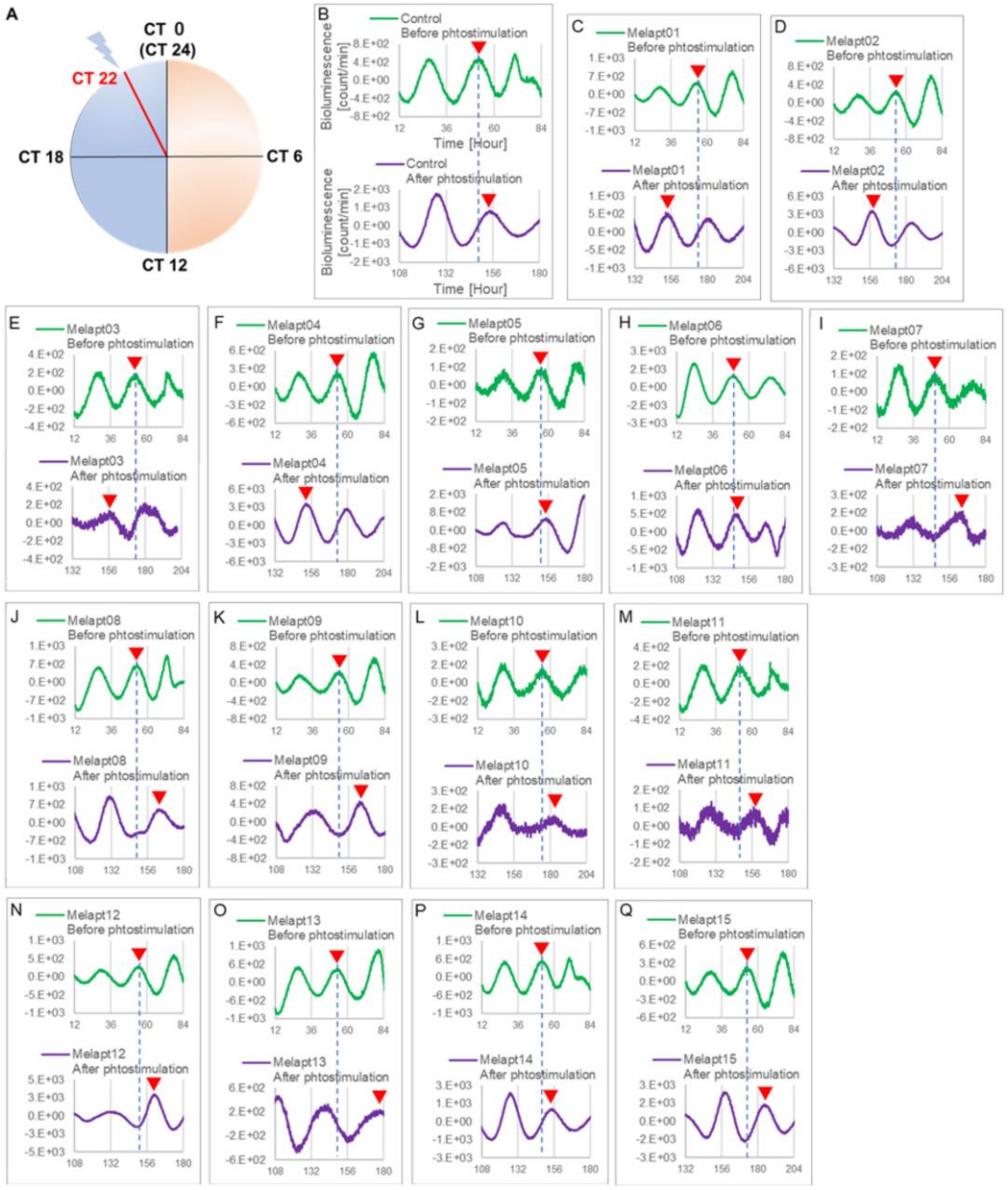

Fig. 3. Title: Changes in *Per2*:ELuc emission rhythms before and after the addition of each Melapt and blue-light photostimulation at CT22

(A) Subjective light period from CT0-12, subjective dark period from CT12-0, and a blue light indicate photostimulation at CT22 of the 24-h clock. (B) *Per2*:ELuc emission upon adding PBS and blue-light photostimulation as a control. (C–Q). *Per2*:ELuc emission upon adding Melapt01-Melapt15 and blue-light photostimulation. Upper row (green), *Per2*:ELuc emission before the addition of Melapt observed for 3 d. Lower row (purple), *Per2*:ELuc emission after addition of Melapt and blue-light photostimulation observed for 3 d. A red triangle indicates the peak. Bioluminescence traces in Figure 3 were estimated from an individual sample. The peak bioluminescent emission rhythms monitored over 3 d before the addition of Melapts and photostimulation (green) are denoted by a dotted line to allow comparison with the peak rhythms in the lower graphs (plotted post-stimulation and colored purple).

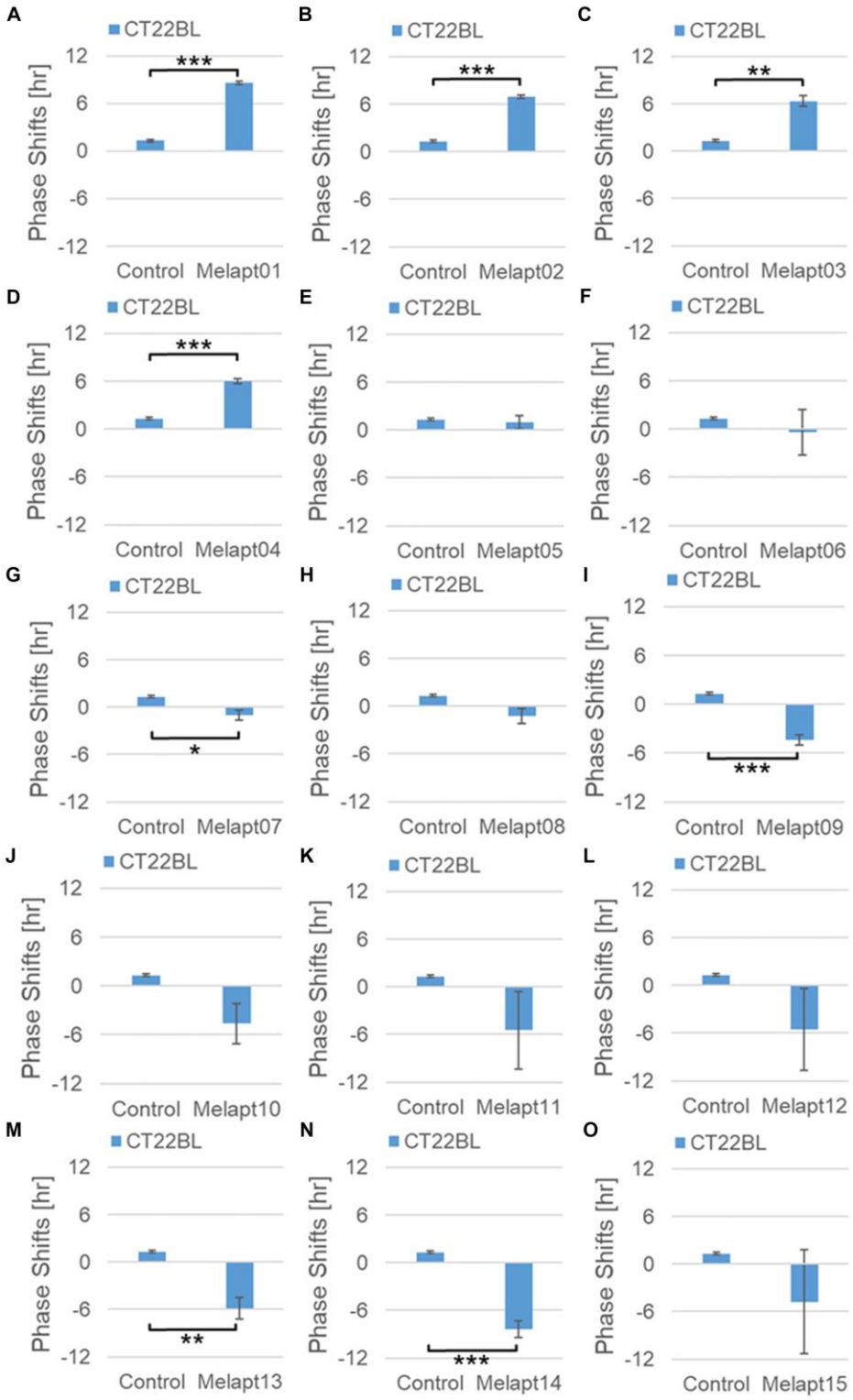

Fig. 4. Phase shift due to binding of Melapts plus photostimulation at subjective dawn (CT22)

(A–O) Phase-shift comparison between Melapts and controls with photo-stimulus at CT22. PBS alone was added to the control cells. The phase shift was calculated from the cosine-fitting curve in Figure 3 using the NINJA program and plotted on a graph. n = 3 (individual samples); *p < 0.05, **p < 0.01, ***p < 0.001, Tukey–Kramer test.

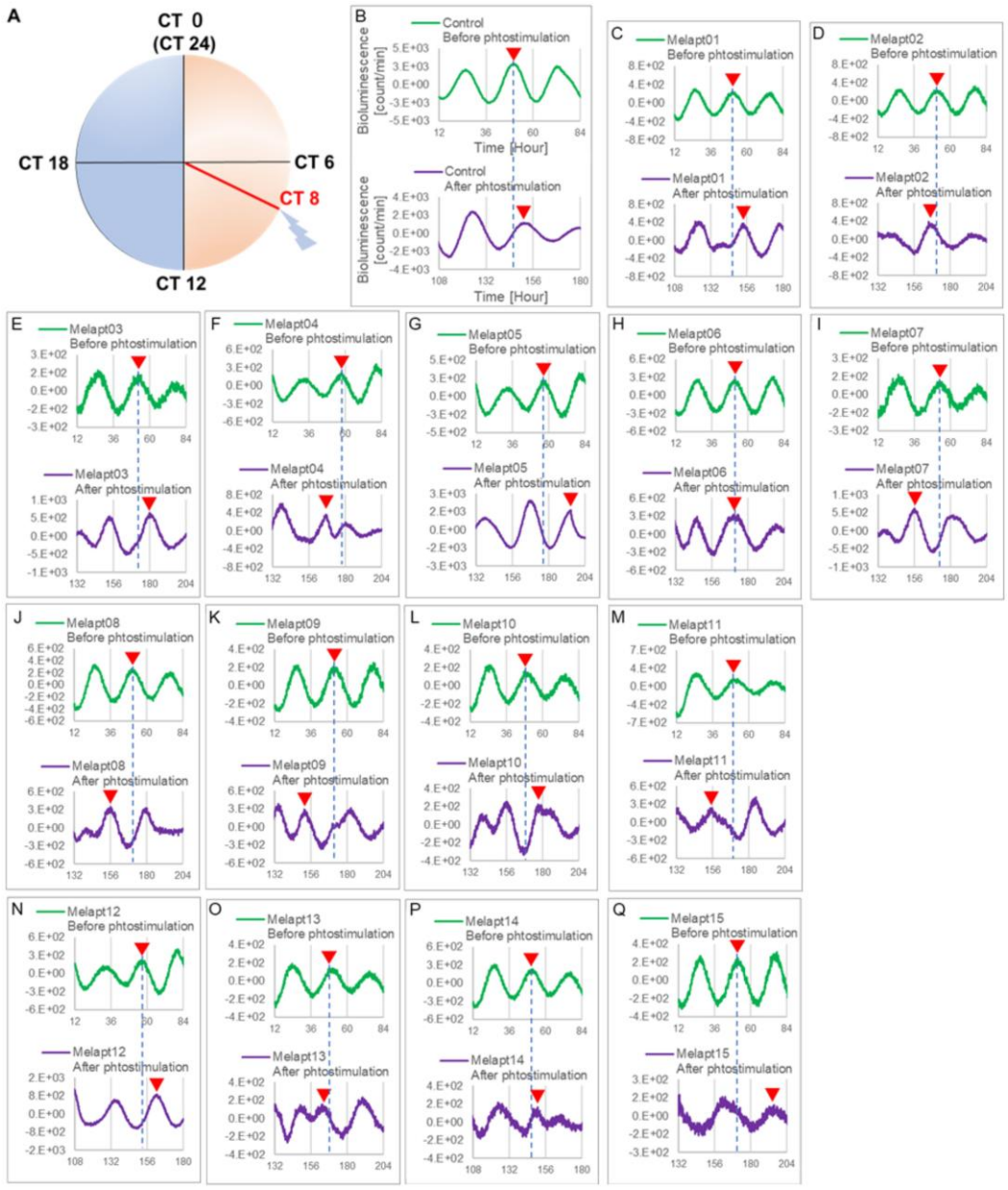

Fig. 5. Changes in *Per2*:ELuc emission rhythms before and after applying Melapts and blue-light photostimulation at CT8

(A) Subjective light period from CT0-12, subjective dark period from CT12-0, and blue light indicate photostimulation at CT8 of the 24-h clock. (B) *Per2*:ELuc emission upon applying PBS and blue-light photostimulation to controls. (C–Q) *Per2*:ELuc emission upon applying Melapt01- Melapt15 and blue-light photostimulation. Upper row (green), *Per2*:ELuc emission before the addition of Melapt observed for 3 d. Lower row (purple), *Per2*:ELuc emission after addition of Melapt and blue-light photostimulation observed for 3 d. Red triangles indicate the peak. Bioluminescence traces in Figure 5 were estimated from an individual sample. The peak of bioluminescent emission rhythms measured over the 3 d before the addition of Melapts and photostimulation (green) is denoted by a dotted line to allow comparison with peak rhythms in the lower graphs (potted post-stimulation).

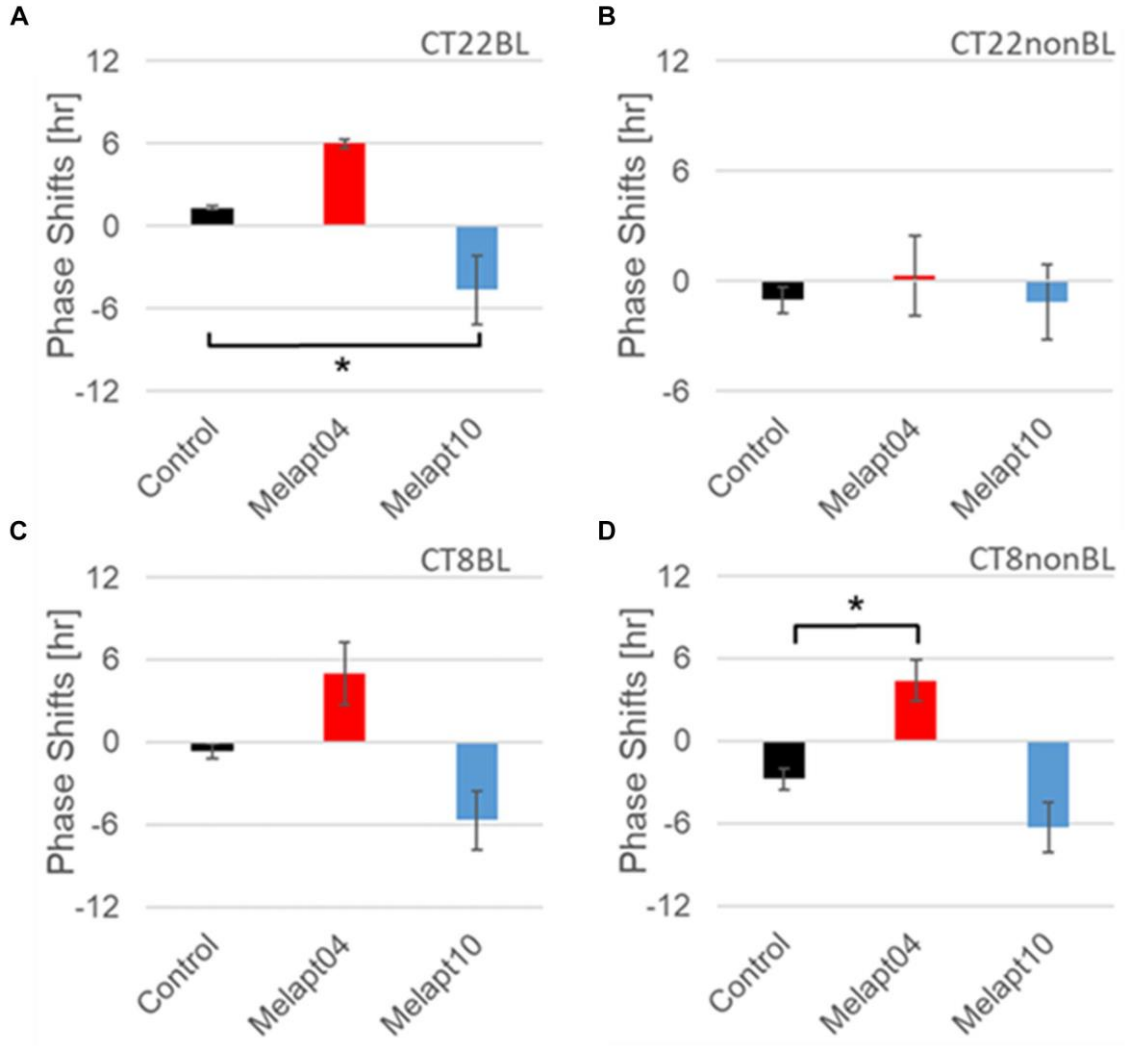

Fig. 6. Similar phase-shift abilities of representative Melapts at both CT22 and CT8

(A) Melapt binding-mediated phase shift induced via photostimulation at subjective dawn (CT22BL). (B) Melapt-mediated phase shift at subjective dawn (CT22nonBL). (C) Melapt binding-mediated phase shifts induced via photostimulation in the afternoon (CT8BL). (D) Melapt-mediated phase shifts in the afternoon (CT8nonBL). Controls showed phase shifts in the absence of Melapts under all conditions. The upper direction shows phase advance, whereas the lower direction shows phase delay. Red: phase advance. Blue: phase delay. *$p < 0.05$, Tukey–Kramer test.

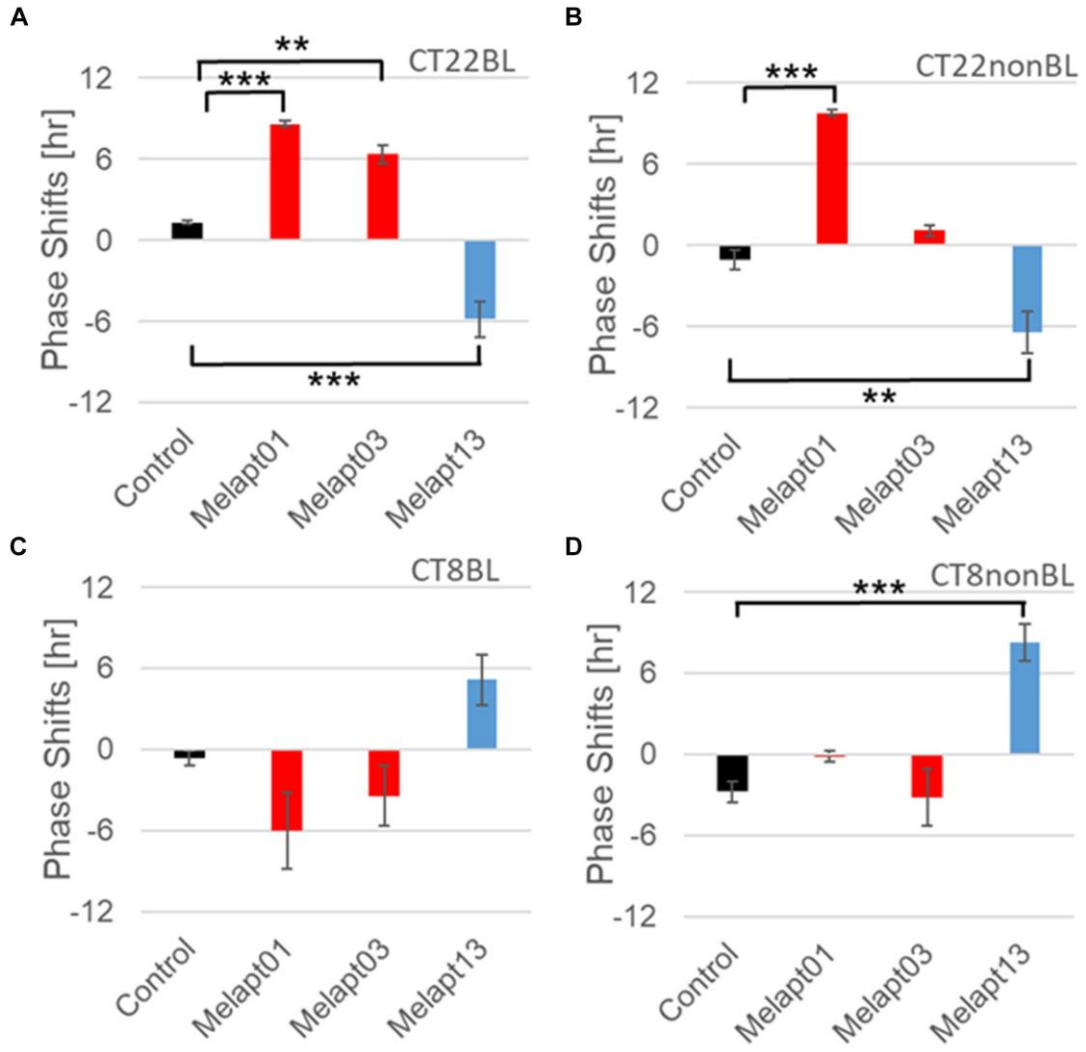

Fig. 7 Reverse-phase shift abilities of representative Melapts at CT22 and CT8

(A) Melapt-mediated phase shift induced by photostimulation at subjective dawn (CT22BL). (B) Melapt-mediated phase shift at subjective dawn (CT22nonBL). (C) Melapt-mediated phase shift induced by photostimulation in the afternoon (CT8BL). (D) Melapt-mediated phase shift in the afternoon (CT8nonBL). Controls were phase shifts without any Melapts under all conditions. The upper direction shows phase advance, whereas the lower direction shows phase delay. Red: phase advance. Blue: phase delay. **$p < 0.01$, ***$p < 0.001$, Tukey–Kramer Test.

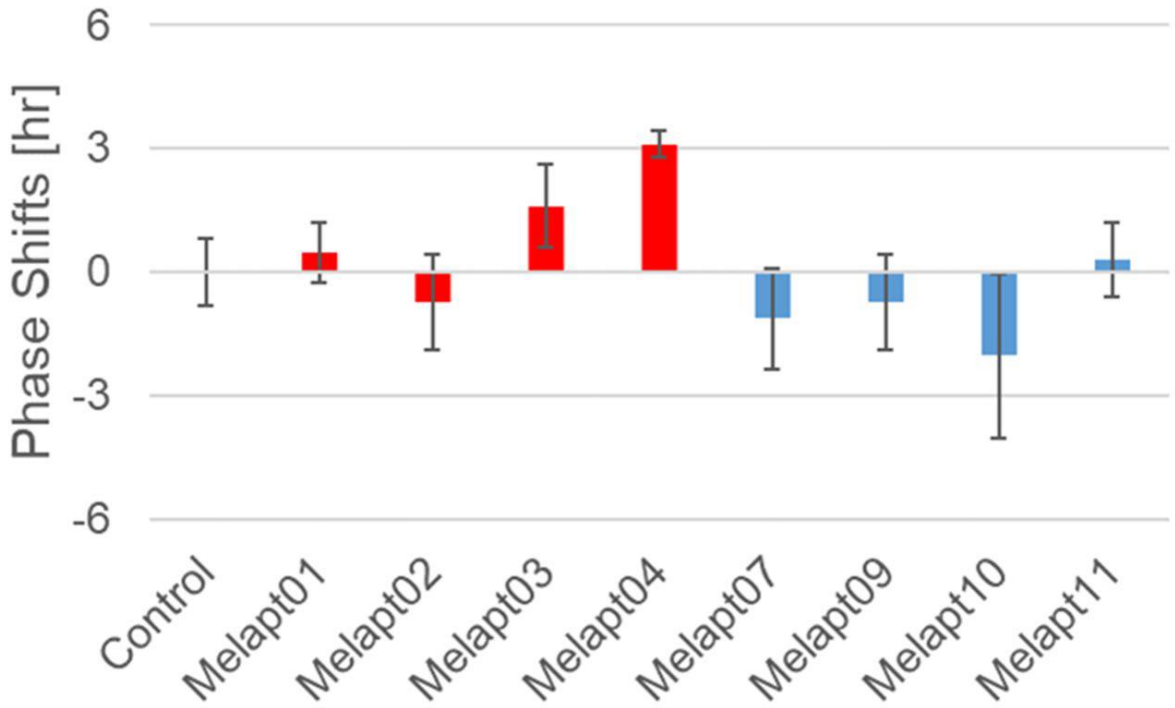

Fig. 8 Phase shift of *Per1*::luc expressional rhythms in SCN slices following injection of Melapts into both bulbus oculi of *Per1*::luc Tg mice

Phase shifts following injection of Melapts into bulbus oculi with LED light stimulus at subjective dawn (CT22). SCN slices were obtained from mice injected with Melapts, and bioluminescence was observed for approximately 5 d. The NINJA program was used to calculate phase shifts. The upper direction shows phase advance, whereas the lower direction shows phase delay. Red: phase advance. Blue: phase delay. n = 3, *$p < 0.05$, Tukey–Kramer test

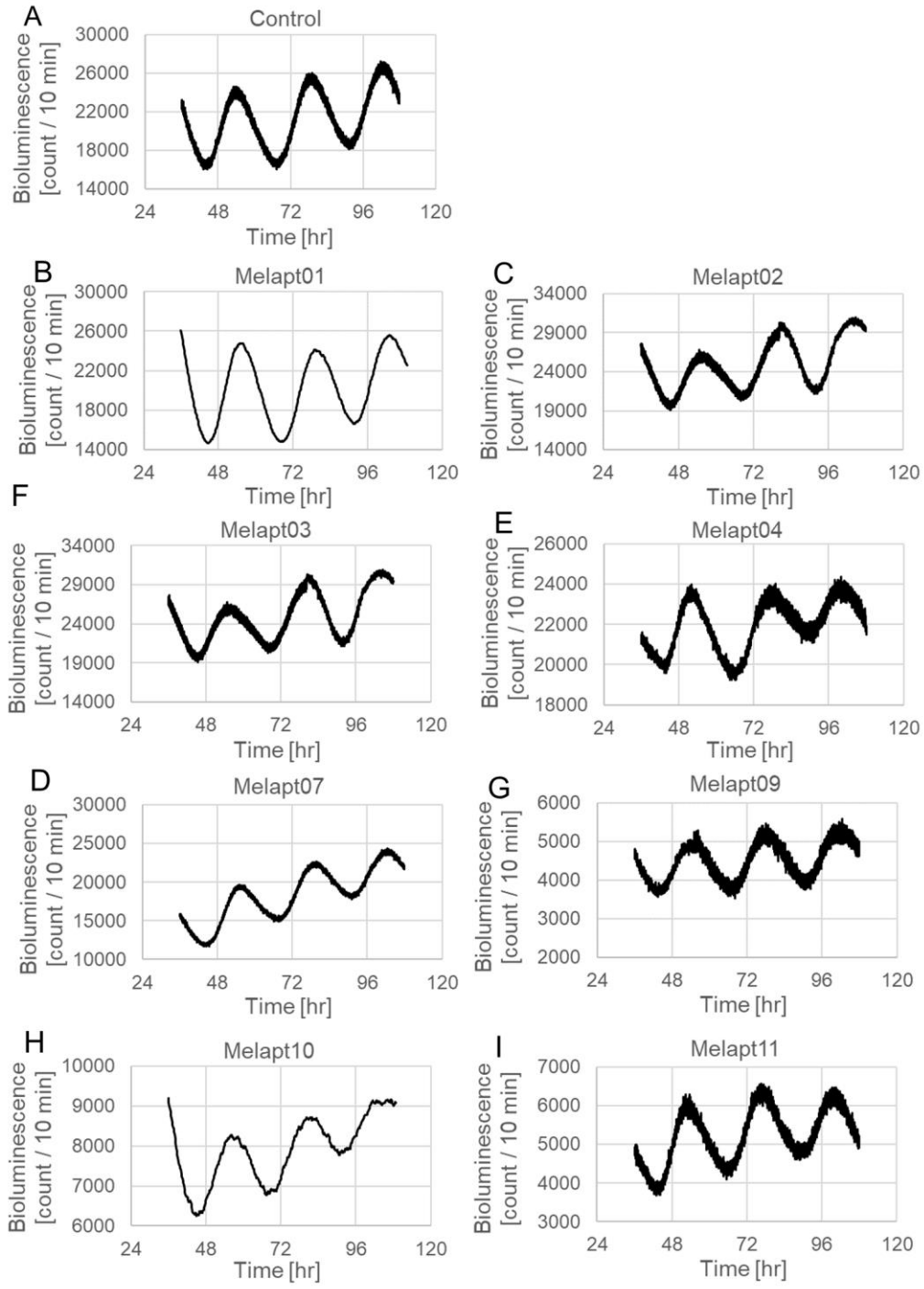

Fig. 9 Phase shifts measured from *Per1*::luc expression rhythms in SCN slices from Melapt-injected *Per1*::lucTg mice

(A) Controls. (B) Injected with Melapt 01. (C) Melapt 02. (D) Melapt 03. (E) Melapt04. (F) Melapt 07. (G) Melapt 09. (H) Melapt 10. (I) Melapt 11. Amplitude was measured at 36−108 h.