

Supplementary Information

DNA damage modulates sleep drive in basal cnidarians with divergent chronotypes

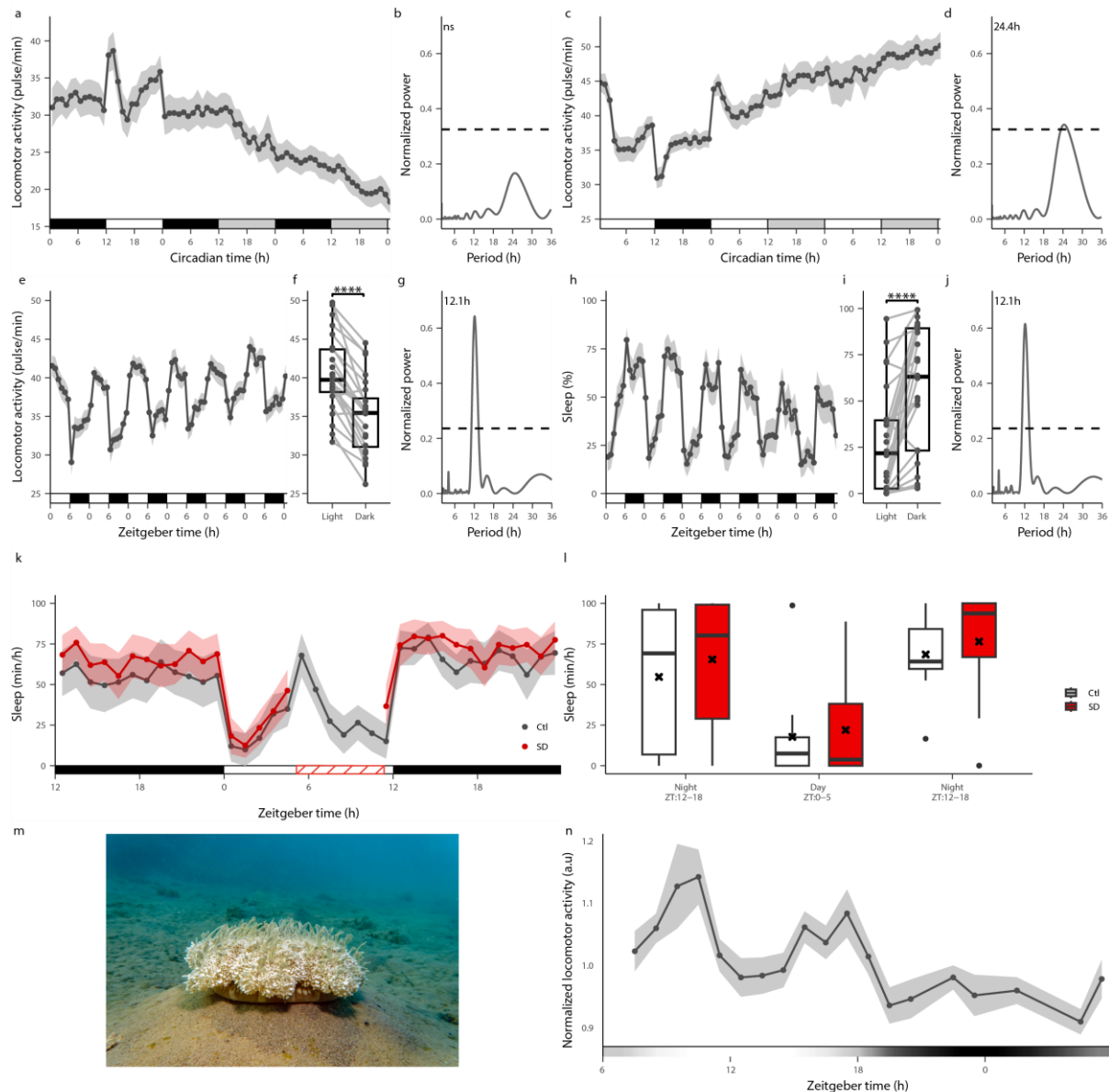
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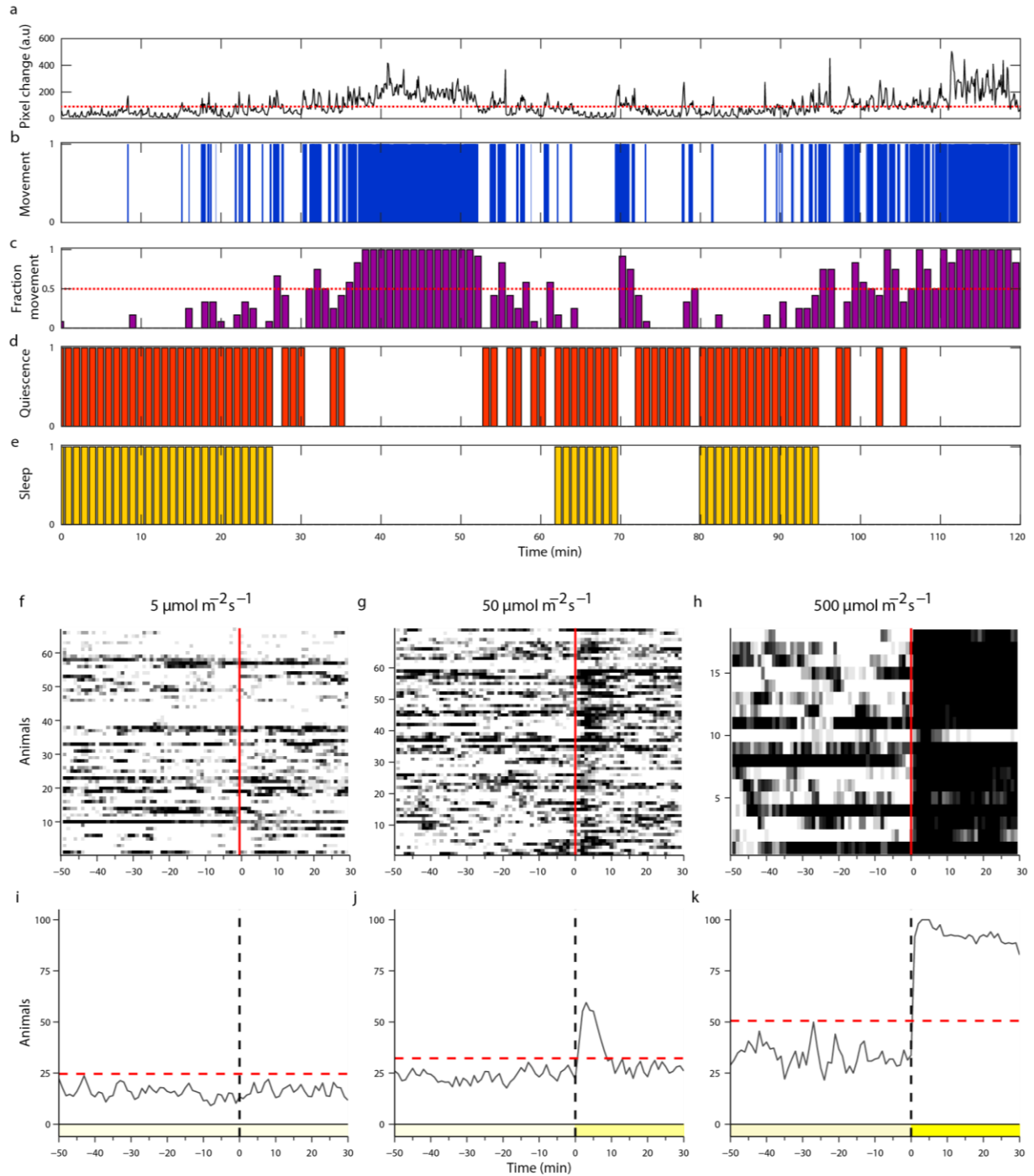
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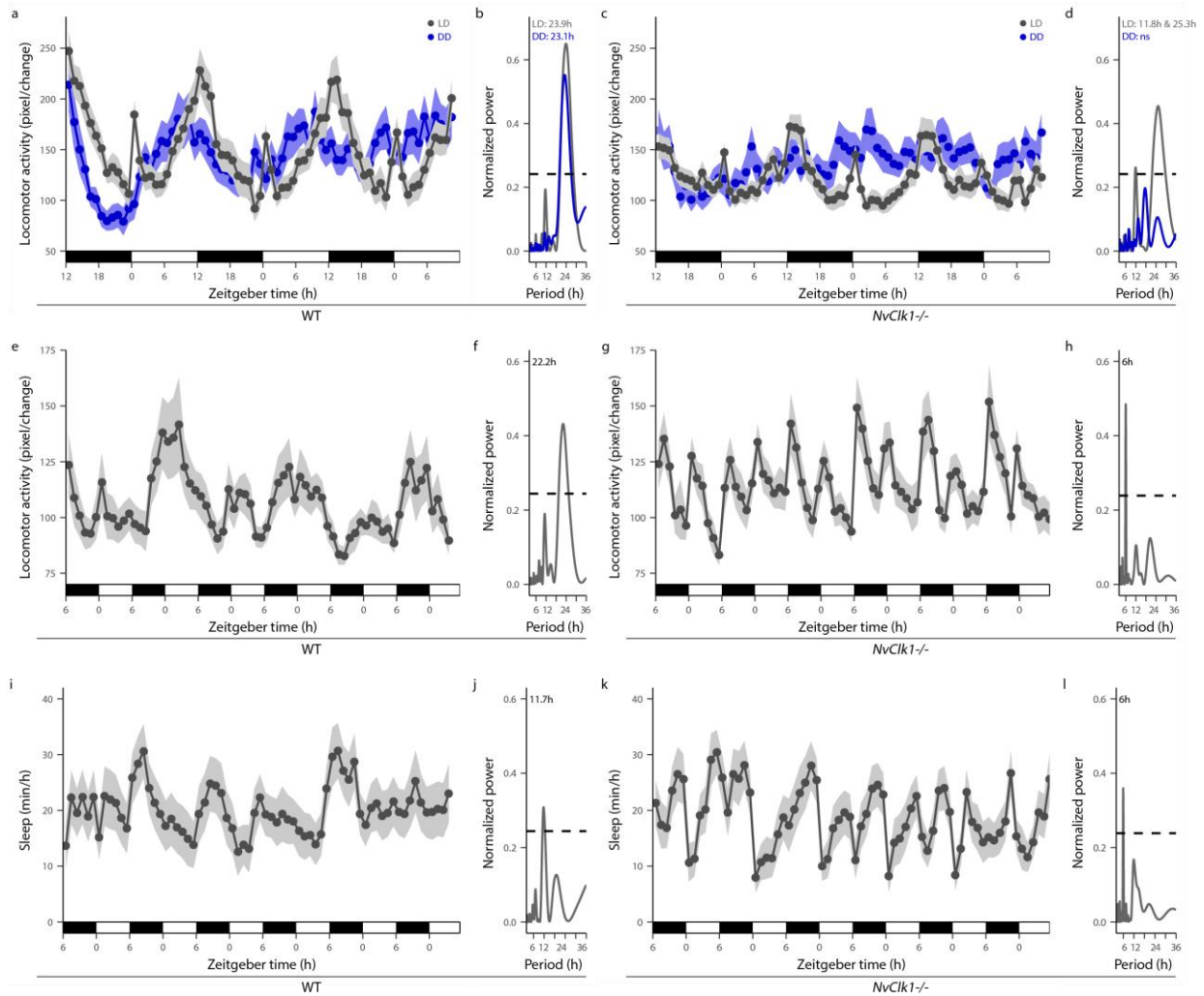
Supplementary Fig. 1. The light/dark cycle control rhythmic locomotor activity in *C. andromeda*. (a) Locomotor activity under DD conditions in *C. andromeda*. (b) LSP-based periodicity detection of the average sleep time shown in (a) ($n=16$). (c) Locomotor activity under LL conditions in *C. andromeda*. (d) LSP-based periodicity detection of the average sleep time shown in (c) ($n=15$). (e) Locomotor activity under LDLD in *C. andromeda* during 72h. (f) Average pulsation rate under LDLD conditions. (g) LSP-based periodicity detection of the average sleep time shown in (e) ($n=24$). (h) Percentage of sleep during 72h under LDLD in *C. andromeda* during 72h. (i) Average sleep time of individuals under LDLD conditions. (j) LSP-based periodicity detection of the average sleep time shown in (h) ($n=24$). (k) Percentage of sleep in control (Ctl; $n=11$) and sleep-deprived (SD; $n=13$) animals under LD. White, black, and red horizontal bars represent day, night, and SD (ZT5-ZT11), respectively. (l) The average percentage of sleep in Ctl and SD animals during the night and day pre-SD, and during night

post-SD. (m) *C. andromeda* in the natural habitat at sea. (n) Normalized average locomotor activity within each bell diameter group, under ambient light and dark (horizontal gray scale bar) in *C. andromeda* at Key Largo, FL ($n=30-40$ animals per time point, total: $n=662$). LSP periodicity detection with $p<0.01$ if period indicated. $p>0.05$ if ns (b, d, g, j). Data in (a, c, e, h, k, n) are presented as mean $\pm$ SEM. Boxplots show median (center line), interquartile range (box), and minima/maxima (whiskers); individual data connected in (f, i); mean (black cross) in (l). Wilcoxon signed-rank test (one-tailed) for (f, i). Mann-Whitney Wilcoxon (two-tailed) for (l). **** $p<0.0001$.

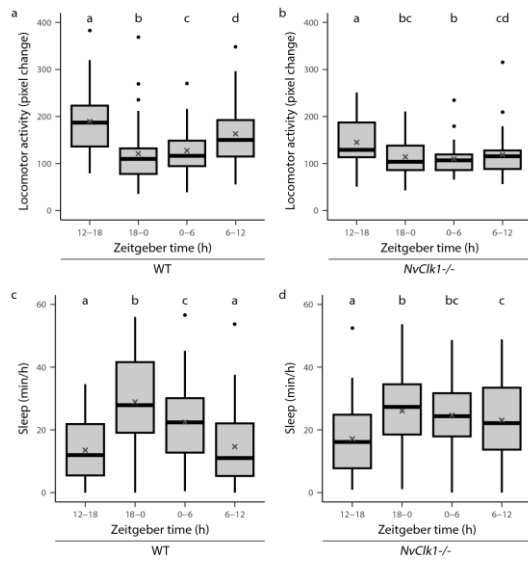


Supplementary Fig. 2. Conversion of locomotor activity to sleep bouts and response to light stimuli in *N. vectensis*. (a) Normalized pixel change tracking during 120min in a single adult *N. vectensis*. The red horizontal dotted line indicates the movement threshold. (b) Conversion and binning of pixel change to movement per minute. (c) Fraction movement per min, red horizontal dotted line indicates the 30sec threshold. (d) If an animal moves less than 30s, the minute is considered quiescence. (e) Based on the arousal threshold experiments presented in Fig. 2, quiescent bout length equal to or longer than 8min is defined as sleep. (f-

h) Arousal threshold experiments: Raster plots show the effect of three light intensities on the movement response of *N. vectensis*. Each horizontal line represents a single *N. vectensis* (white - quiescent, black - active). The red vertical line indicates the transition time to light stimulation. (i-k) Percentage of animals (+5 PPFD: $n=67$; +50 PPFD: $n=67$, +500 PPFD: $n=18$) responding to the three intensities of light stimulation. The red horizontal dashed line indicates the movement threshold.



Supplementary Fig. 3. The hierarchy of circadian and light regulation of locomotor activity and sleep in *N. vectensis*. (a) Locomotor activity during 72h in WT *N. vectensis* under LD (LD: $n=33$) and DD (DD: $n=24$). (b) LSP-based periodicity detection of the average locomotor activity in WT *N. vectensis* under LD and DD. (c) Locomotor activity during 72h in *NvClk*^{Δ/Δ} under LD (LD: $n=34$) and DD (DD: $n=26$). (d) LSP-based periodicity detection of the average locomotor activity in *NvClk*^{Δ/Δ} under LD and DD. (e) Locomotor activity during 72h in WT *N. vectensis* under LDLD ($n=24$). (f) LSP-based periodicity detection of the average locomotor activity in WT under LDLD. (g) Locomotor activity during 72h in *NvClk*^{Δ/Δ} under LDLD ($n=24$). (h) LSP-based periodicity detection of the average locomotor activity in *NvClk*^{Δ/Δ} under LDLD. (i) Sleep time during 72h in WT *N. vectensis* under LDLD ($n=24$). (j) LSP-based periodicity detection of the average sleep time in WT under LDLD. (k) Sleep time during 72h in *NvClk*^{Δ/Δ} under LDLD ($n=24$). (l) LSP-based periodicity detection of the average sleep time in *NvClk*^{Δ/Δ} under LDLD. LSP periodicity detection with $p < 0.0001$ if period indicated. $p > 0.05$ if ns for panels (b, d, f, h, j, l). Data in (a, c, e, g, i, k) are presented as mean $\pm$ SEM.



Supplementary Fig. 4. Average locomotor activity and sleep in 6h bin. (a, b) Average locomotor activity and (c, d) average sleep time across four 6h time bins (ZT12-18, ZT18-ZT0, ZT0-ZT6, and ZT6-12) under LD in WT (a, c) and *NvClk* ^{Δ/Δ} (b, d) *N.vectensis* (WT: $n=33$, *NvClk* ^{Δ/Δ} : $n=34$). Boxplots show median (center line), interquartile range (box), and minima/maxima (whiskers); individual data connected in (f, i), and mean (black cross). Different lowercase letters above the boxplots indicate statistically significant differences (Pairwise Wilcoxon signed-rank test, two-tailed; Bonferroni controlled; $p < 0.05$).