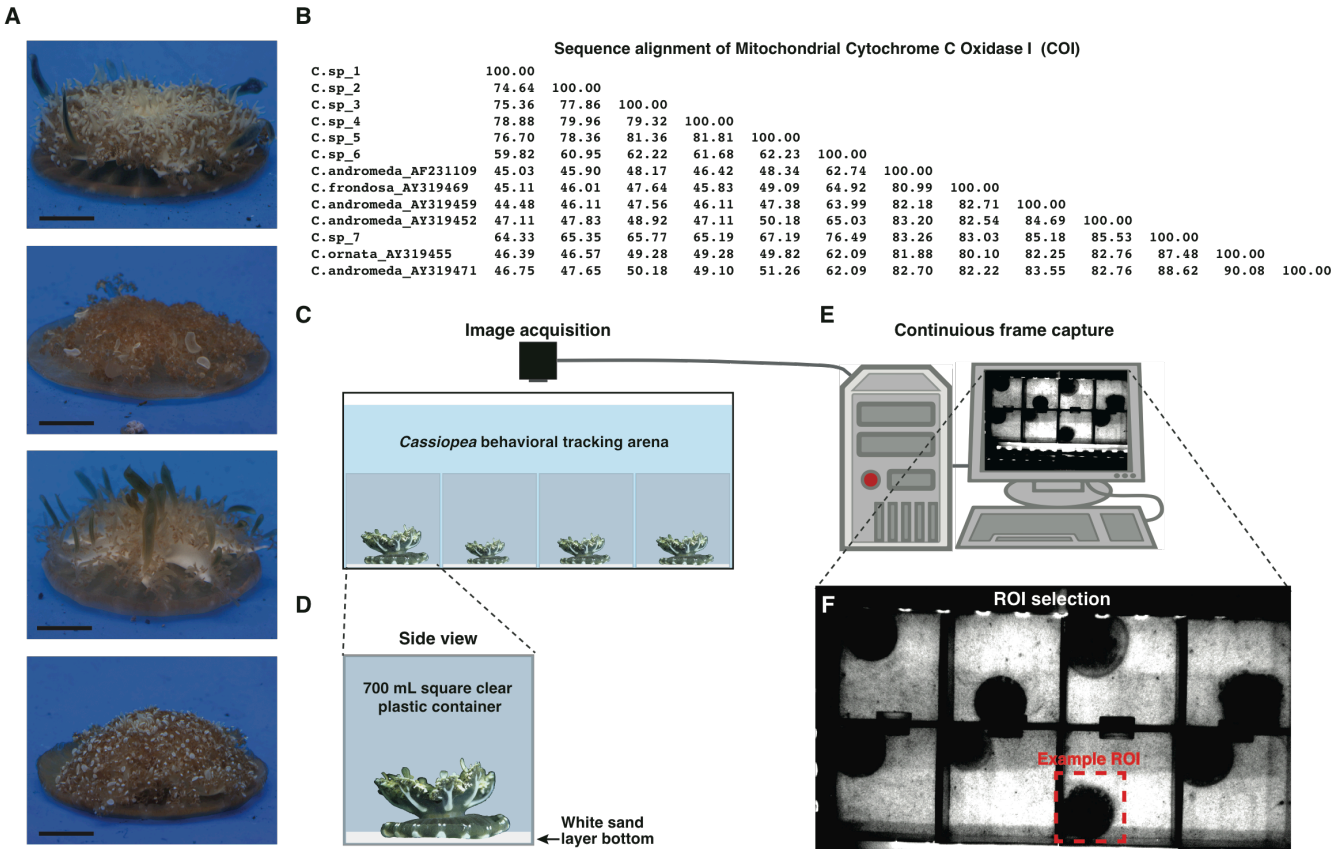

Figure S1. *Cassiopea spp.* diversity and behavioral tracking system, Related to Figure 1. (**A**) Images of four *Cassiopea spp.* with different morphology (scale bar 1 cm). This is representative of the range of morphologies used in the experiments. (**B**) Percent amino acid identity matrix comparing mitochondrial cytochrome C oxidase I (COI) amino acid sequences of seven *Cassiopea spp*. used in this study (C.sp_1 – C. sp_7) with six previously described *Cassiopea spp.* (Taxon_GeneBank number). (**C**) For the behavioral tracking system jellyfish were placed in behavioral tracking arenas with cameras recording from above. (**D**) Each jellyfish was placed in a clear, plastic container with white sand layering the bottom. The white sand provides contrast, allowing better behavioral tracking. (**E**) Images were captured at a rate of 15 frames per second and saved directly onto solid-state hard drives. (**F**) A region of interest (ROI) around each jellyfish was selected for downstream processing.

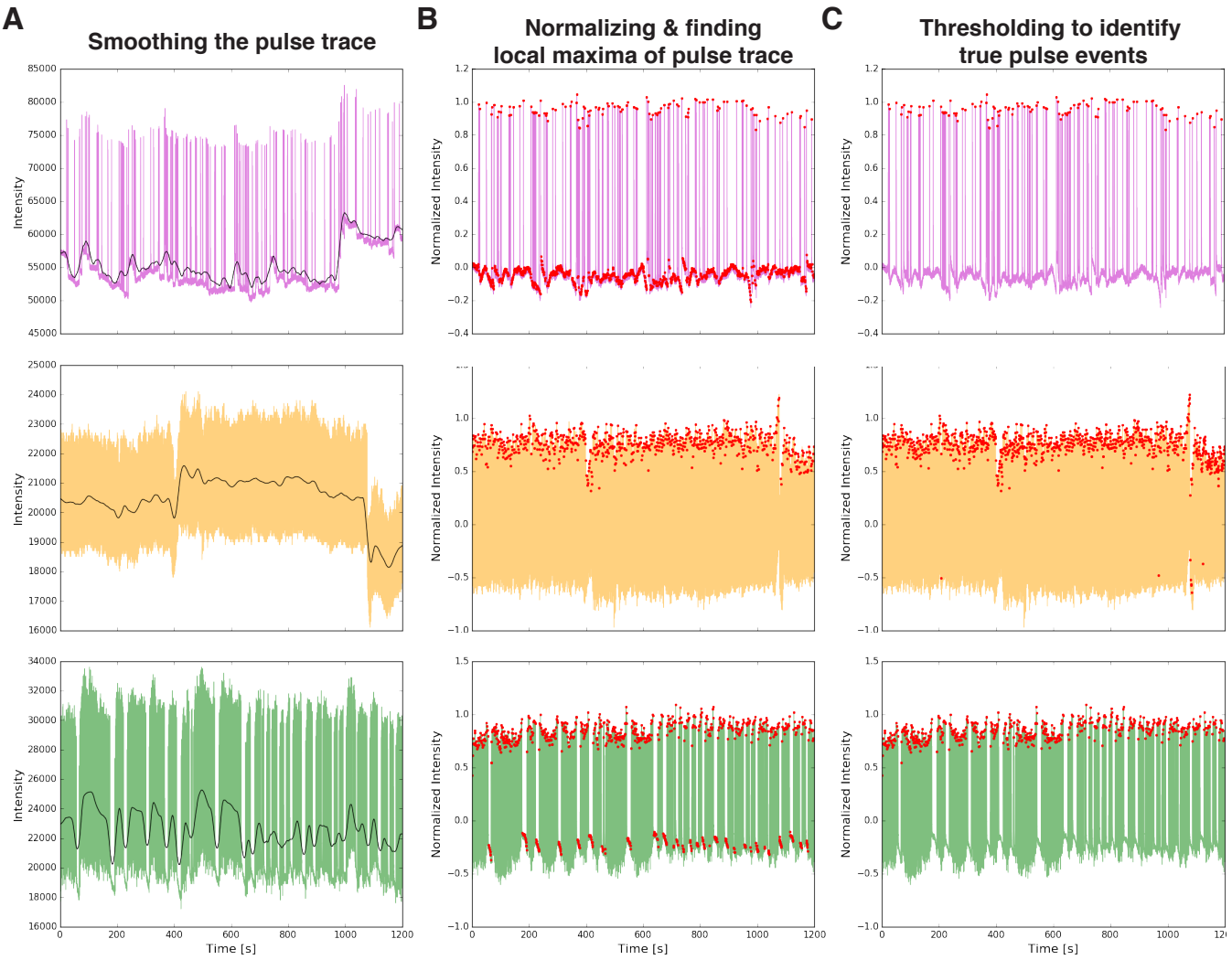

Figure S2. Processing the jellyfish pulse-trace data to count pulse events, Related to Figure 1. Each color represents data from a different jellyfish (pink, orange, and green). (**A**) Smoothing the pulse-trace for normalization. Black line represents the smoothed trace for a 20 min recording. (**B**) Normalized pulsing traces for three different jellyfish with local maxima indicated by red dots. Many local maxima are detected within pauses in activity due to noise (small fluctuations in intensity), which are removed by thresholding. (**C**) Thresholding to identify local maxima at pulsing peaks. Pulsing peaks are indicated by red dots. For more details see the '*Cassiopea* behavioral tracking' section of the **STAR Methods**.

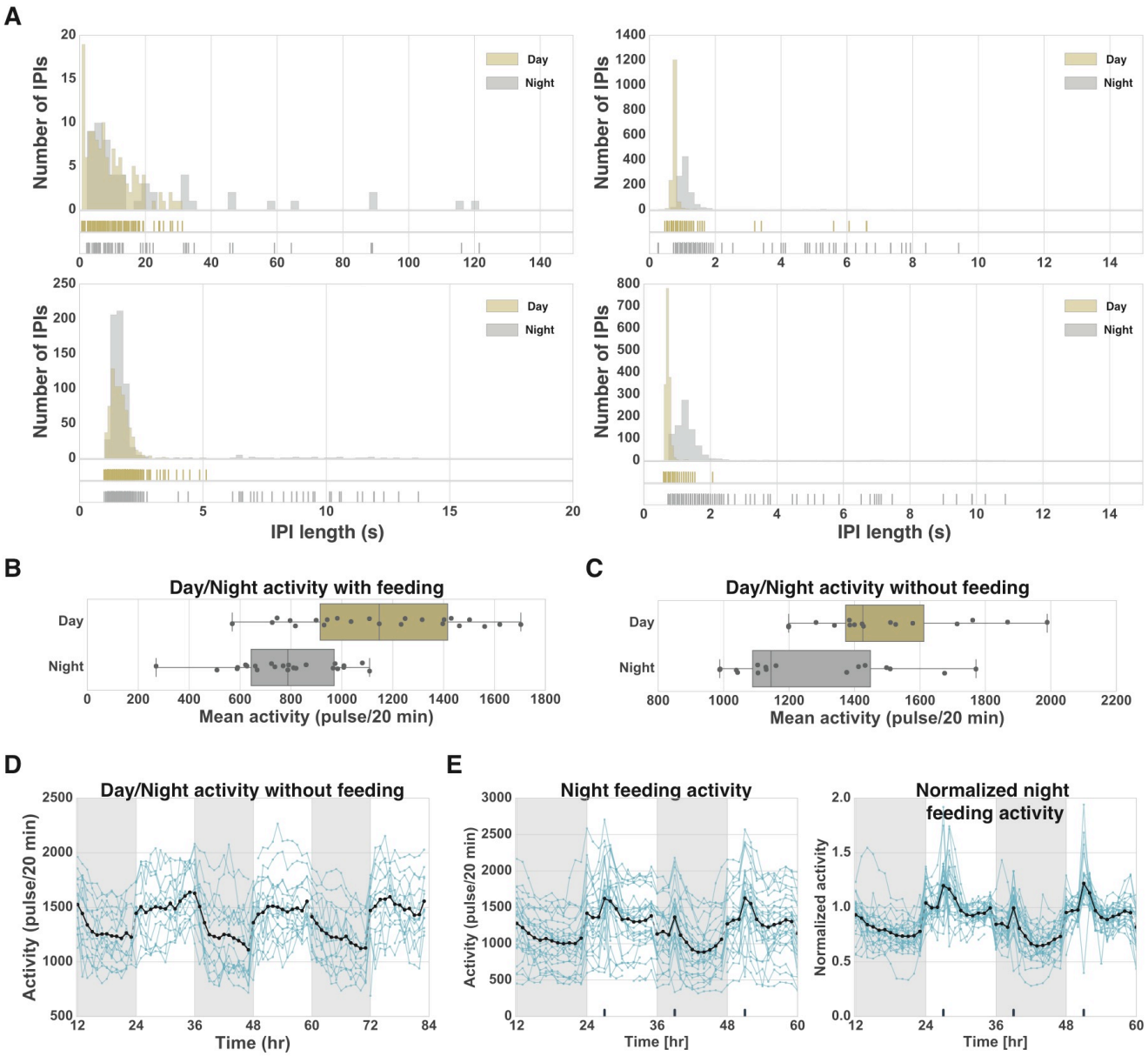

Figure S3. *Cassiopea* pulsing quiescence at night, Related to Figure 2. (**A**) Distribution of IPI length for four *Cassiopea* during the day (yellow) and night (gray) showing each IPI event. Tick marks below the distributions show each IPI length during the day (yellow) and night (gray). The ticks highlight the long-pauses that are more common at night for all jellyfish (**Data S1**). Box plot of *Cassiopea* day and night pulsing activity with feeding (**B),** and without feeding (**C**). Each dot represents a single jellyfish, mean activity is calculated over 6 (feeding, **B**) or 3 (without feeding, **C**) days and nights. For **D** and **E** each blue line corresponds to a single jellyfish. The black line indicates the mean activity of all jellyfish. Dark gray shading indicates night periods. (**D**) Day and night activity of *Cassiopea* without feeding. Baseline activity (pulses/20 min) without feeding of 16 jellyfish tracked over three days. (**E**) Feeding induced arousal rapidly reverses the night quiescent state. Dark tick marks on x-axis indicate time of feeding. Activity (pulses/20 min) and normalized activity of 30 jellyfish tracked over two day/nights from six laboratory replicates. Jellyfish were fed 4 hours into each day and 4 hours into the second night.

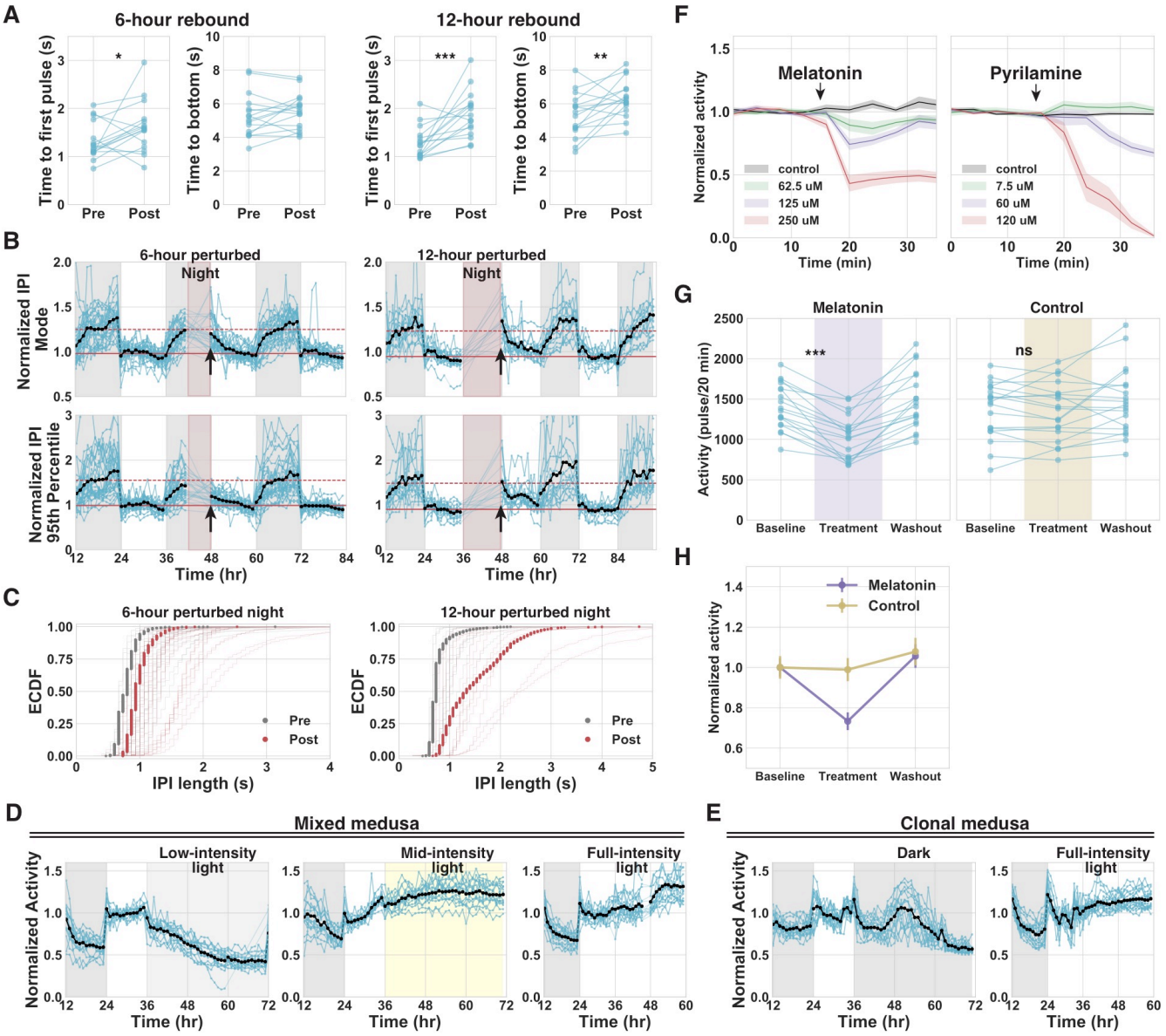

Figure S4. Regulation of quiescence in *Cassiopea*, Related to Figure 4. Each blue line corresponds to a single jellyfish. The black line indicates the mean activity of all jellyfish. Dark gray shading indicates night periods. (**A**) Sensory responsiveness was tested during periods of decreased activity before (pre) and after (post) either the 6-hour or 12-hour perturbation periods (10 s water pulses every 20 min) using the assay described in **Figure 3**. Time to first pulse after drop and time to reach bottom after drop were measured during the day pre or post perturbation. After perturbation (post), an increased response latency was observed. Two-sided paired *t*-test, pre versus post, $*P<5x10^{-2}$, $**P<10^{-2}$, $***P<10^{-3}$. (**B**) Maroon horizontal lines show the mean activity of pre-perturbation day (solid) and pre-perturbation night (dashed). Maroon shading indicates perturbation periods with 10 s water pulses every 20 min. In these experiments jellyfish were exposed to different perturbation lengths (either 6 or 12 hours) during the night. Plotted here is the normalized mode and 95th percentile of the IPI length for all jellyfish tracked over multiple days. Perturbation of either 30 jellyfish for the last 6 hours of the night or 16 jellyfish for an entire 12-hour night. For both the 6-hour and 12-hour perturbation there is an increase in the mode and 95th percentile of the IPI length after perturbation (black arrowhead). (**C**) Empirical cumulative distribution function (ECDF) of daytime IPI length for all jellyfish pre (gray) and post (maroon) perturbation (thin lines, single jellyfish; dots, all jellyfish). Jellyfish exhibited increased IPI lengths after perturbation compared to before perturbation. These results suggest that the increased quiescence observed in **Figure 4** results from both a decreased frequency of pulsing and an increase in the length of pause events. (**D-E**) Monitoring activity with different light or dark conditions suggests that nighttime quiescence may be under circadian regulation. (**D**) Prolonged light exposure of *Cassiopea* shows no circadian cycling. 16 jellyfish were exposed

to either 36-hours of continuous low-intensity light (light-gray shading) from hour 36 to hour 72, 36-hours of continuous mid-intensity light (yellow shading) from hour 36 to hour 72, or 36-hours of continuous full-intensity light from hour 24 to hour 60. Each experiment represents two laboratory replicates using a mixed population of *Cassiopea spp*. (**E**) Prolonged exposure to dark conditions of jellyfish shows circadian cycling when using a clonal population of medusa (*Cassiopea xamachana*), see **STAR Methods**. 16 jellyfish were exposed to dark conditions from hour 36 to hour 72 or full-intensity light from hour 24 to hour 60. With this clonal population of jellyfish, circadian cycling of behavior is only observed for constant dark conditions and not constant full-intensity light conditions, consistent with results seen in the mixed population of *Cassiopea* shown in (**D**). (**F-H)** *Cassiopea* exhibit a decrease in activity in response to melatonin and pyrilamine exposure during the day. (**F**) Treatment with either pyrilamine or melatonin effects pulsing activity. The colored lines represent different concentrations of compounds tested. Activity was monitored before and after treatment. Time of treatment is indicated by a black arrow. Both melatonin and pyrilamine induce a concentration-dependent decrease in pulsing activity. (**G**) Activity of 18 *Cassiopea* exposed to 125 μM melatonin solubilized in ethanol compared to 19 *Cassiopea* treated with ethanol vehicle control from four laboratory replicates. *Cassiopea* were monitored for 20 min before (baseline), during (treatment), and after (washout) either melatonin or vehicle treatment. Two-sided paired *t*-test, before/during melatonin treatment: $P = 4\text{x}10^{-7}$, and before/during vehicle treatment: $P = 7\text{x}10^{-1}$. ***$P<10^{-3}$, ns not significant (ns) $P>5\text{x}10^{-2}$. (**H**) Comparison of the normalized mean activity between the melatonin and control treatment. Error-bars represent the standard error of the mean.