Supplementary Figure Legends:

Supplementary Figure 1. Monitoring of 2nd and 3rd instar larvae

The LarvaLodge can be utilized for long term behavioral experiments using 2nd instar larvae (green box) or short term using 3rd instars (red box). Scale bar = 5 mm.

Supplementary Figure 2. Preparing the LarvaLodge

(a) Image of a LarvaLodge. Wells were filled with 3% agar, 2% Sucrose media. Well diameters are 11 mm across. (b) Magnified image of two wells after yeast paste was applied to the surface. Scale bar = 5 mm.

Supplementary Figure 3. UV Treatment of Worms

To protect the untreated controls on the WorMotel, we use a combination of a piece of folded paper and aluminum foil tent. (a) Paper is used under the aluminum foil because UV rays could bounce off of aluminum foil alone and still reach worms underneath. The folded piece of paper fits on the WorMotel chip to cover as many rows of the chip as desired. (b) Side view shows how the folded piece fits down in between rows of wells to hold it securely in place. (c) View from above the chip of where the aluminum foil sits, folded to fit over the paper. (d) Side view of the placement of the aluminum foil tent. (e) View of the paper and aluminum foil from the

open end; the aluminum foil tent fits over the paper loosely so that putting the aluminum foil tent on does not disrupt the paper.

Supplementary Figure 4. Image exposure range

Examples of (a) optimally exposed, (b) overexposed, and (c) underexposed images of a WorMotel. Well centers are spaced 4.5 mm apart.

Supplementary Video 1: Continuous monitoring in a WorMotel of a single wild-type *C. elegans* hermaphrodite from the embryo stage to the adult stage. The newly-hatched first larval stage animal is about 200 μm long and the adult animal is about 1000 μm long. The well diameter is 3.5 mm.

Supplementary Video 2: Monitoring pharyngeal pumping of a single adult *C. elegans* hermaphrodite by increasing the magnification. The adult worm is about 1200 μm long.

Supplementary Data:

This zip file includes the following folders:

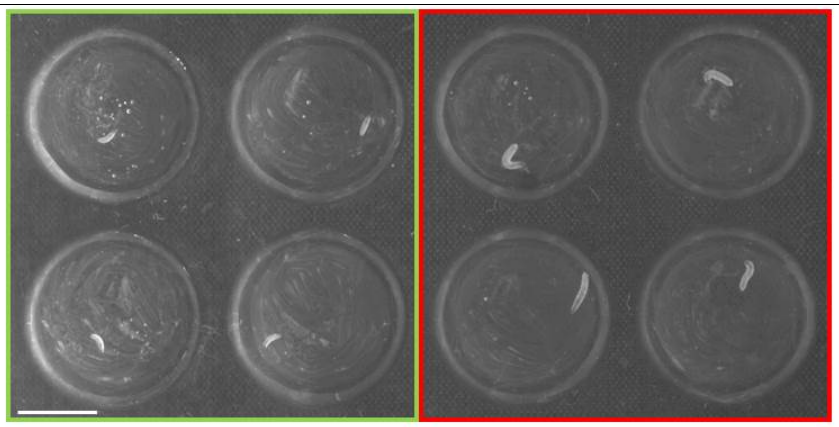
blue_light_setup: Contains a simple Matlab script to control an LED via a Nidaq Input/Output device. This folder also contains a Nidaq Matlab plugin which is required to establish a connection between Matlab and Nidaq.

Device fabrication: Contains .stl files for the WorMotel and LarvaLodge which can be directly sent to a 3D printer for printing. This folder also contains Matlab scripts which can generate the .stl files for users to design custom molds.

drosophila: Contains graphical user interface (GUI) for acquiring images of *Drosophila* larvae, an example analysis of LarvaLodge quiescence data, and scripts for open-loop and closed-loop sleep deprivation.

worm: Contains graphical user interface (GUI) for acquiring images of *C. elegans* and behavioral analysis scripts.

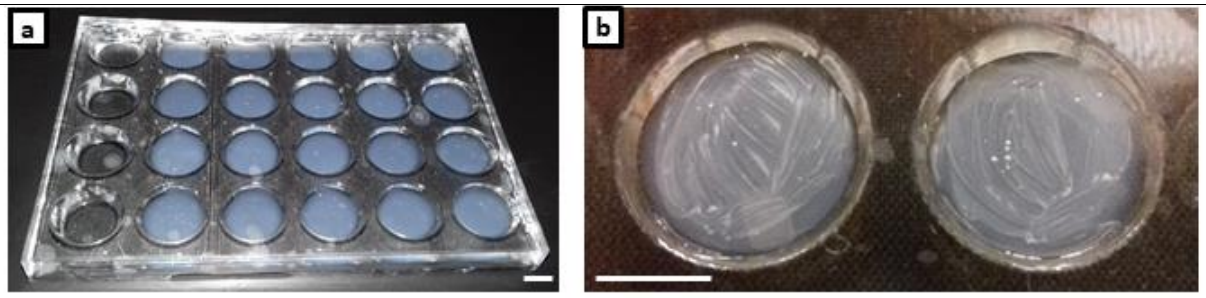
LightBox_Checklist: Contains a list of parts to construct the blue LED apparatus.



Supplementary Figure 1

Monitoring of 2nd and 3rd instar larvae

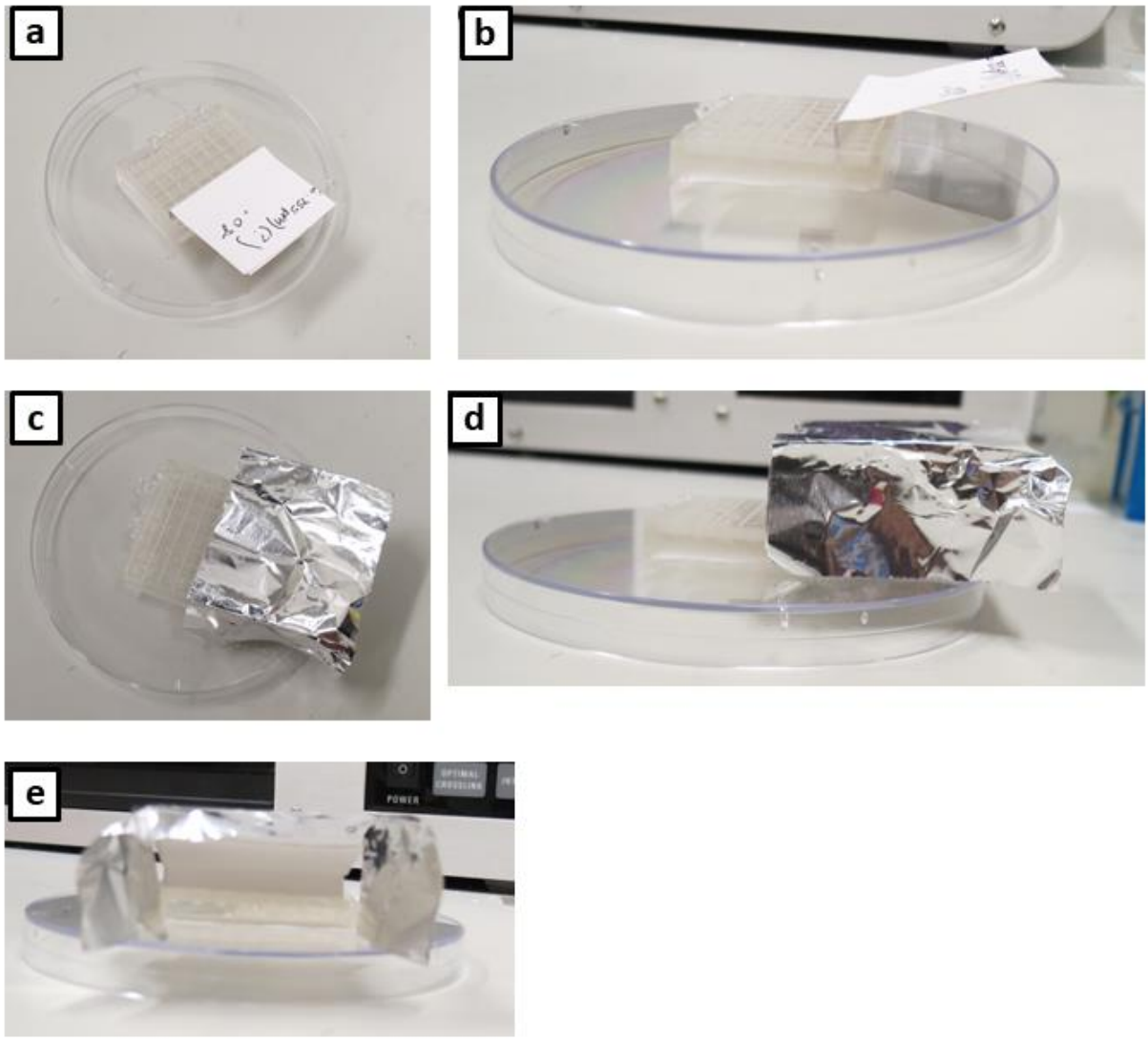
The LarvaLodge can be utilized for long term behavioral experiments using 2nd instar larvae (green box) or short term using 3rd instars (red box). Scale bar = 5 mm.



Supplementary Figure 2

Preparing the LarvaLodge

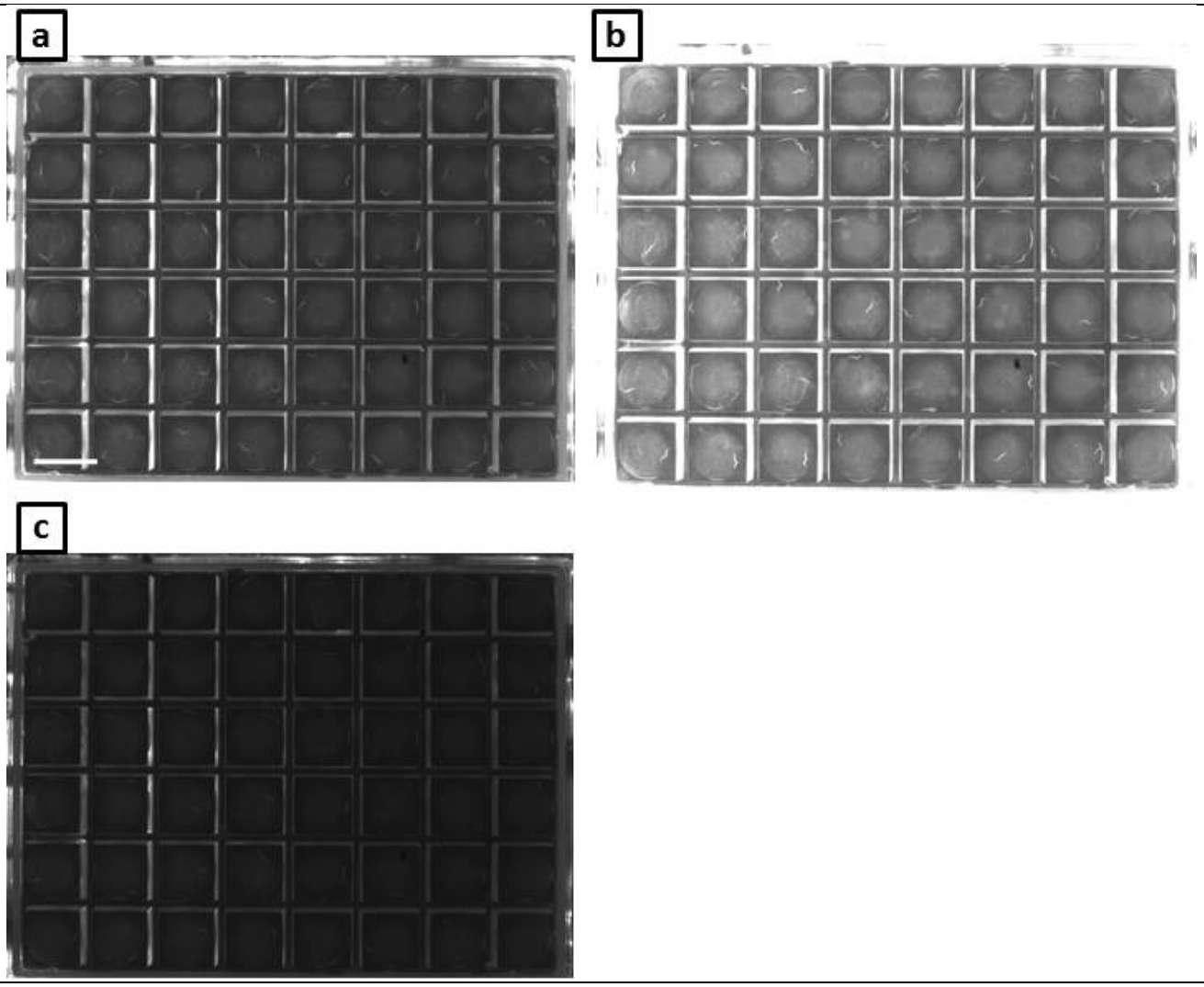
(a) Image of a LarvaLodge. Wells are filled with 3% agar, 2% Sucrose media. Well diameters are 11 mm across. (b) Magnified image of two wells with yeast paste applied to the surface. Scale bar = 5 mm.



Supplementary Figure 3

UV treatment of worms

To protect the untreated controls on the WorMotel, we use a combination of a piece of folded paper and aluminum foil tent. (a) Paper is used under the aluminum foil because UV rays could bounce off of aluminum foil alone and still reach worms underneath. The folded piece of paper fits on the WorMotel chip to cover as many rows of the chip as desired. (b) Side view shows how the folded piece fits down in between rows of wells to hold it securely in place. (c) View from above the chip of where the aluminum foil sits, folded to fit over the paper. (d) Side view of the placement of the aluminum foil tent. (e) View of the paper and aluminum foil from the open end; the aluminum foil tent fits over the paper loosely so that putting the aluminum foil tent on does not disrupt the paper.



Supplementary Figure 4

Image exposure range

(a-c) Examples of (a) optimally exposed, (b) overexposed, (c) underexposed WorMotel chip. Scale bar = 5mm.