Supplementary Methods

Strains

C. elegans strains were cultured according to standard techniques[1]. Strains generated in this study include ERL25: ttx-1(p767) I; zIs356 IV (cross of strains TJ356 and PR767), ERL26: tax-4(p678) III; zIs356 IV (cross of strains TJ356 and PR678), ERL27: ttx-3(ks5) X; zIs356 IV (cross of strains TJ356 and FK134), ERL31: jkk-1(km2) X; zIs356 IV (cross of strains TJ356 and KU2), ERL42: ERL54: ceh-36(ks86) X; zIs356 IV (cross of strains TJ356 and FK311), and ERL56: unc-3 (e151) X; zIs356 IV (cross of strains TJ356 and CB151). Other strains include N2, TJ356: zIs356[P_{daf-16}::DAF-16a/b::GFP+rol-6^{D}]IV, and muEx108[P_{daf-16}::DAF-16a::GFP/b^{KO}]; ocr-2(yz5) IV (a gift from JY Sze). Some of these strains were provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440). Mutations in all these strains were verified by sequencing.

Microfluidic device

Design

The microfluidic device used in this study is composed of double layer microfluidic channels separated by a thin (100 μm) PDMS membrane (**Supporting Methods Fig. 1a**). The 50μm-thick channels in the first layer were used to comfortably accommodate the worms and to deliver food. The design of this layer follows the one described previously[2]. The second layer, designed to control the temperature of the worms immediately underneath, had either a single wide (5 mm) and deep (300μm) channel (**Fig. 1a** of the main text), or two channels of similar dimension separated by a PDMS membrane (200μm) (**Supporting Methods Fig. 1b**).

Fabrication

Photo masks were prepared with AutoCAD and printed at 20,000 dpi resolution (CAD/Art Services). The masks of the first and second layers were patterned on 3-inch wafers (Silicon Quest) using SU8 3050 and SU8 3250 photoresists (Microchem), with a thickness of 50 μm and 300 μm respectively. Molds were then prepared using standard protocol.

Polydimethylsiloxane (PDMS) was spin coated on the first layer mold with a thickness of 150 μm, and poured on the second layer mold (8 mm thickness). The PDMS molds were then transferred to an oven (65°C, 2 hours). PDMS of the second layer was peeled off the mold. Inlet\outlet holes and a thermocouple socket were drilled with a 1.07-mm dermal punch (Harris Uni-corn 0.75). The second layer PDMS was irreversibly bonded to the first layer PDMS (which is still attached to its silicon mold) by plasma activation, and then kept for 2 hours at 65°C before removal of the PDMS from the silicon mold. Inlet and outlet holes of the first layer channels were drilled through the whole device. The microfluidic device was then irreversibly bonded to a pre-cleaned glass slide (1″X3″) by plasma activation and kept for another 2 hours at 65°C.

Temperature control

A fast laminar flow (1.5 ml/min) of 2.5% Pluronic F127 solution (Sigma) was streamed through the second layer channel. The polymer solution acts as a surfactant; it adsorbs to the surface and prevents bubbles from adhering the PDMS. Fluid temperature was raised by immersing the Pluronic solution tubing in a hot bath (65°C). The precise temperature level of fluid streamed through the temperature control channel was adjusted

by controlling the length of the tube between the device and the hot bath. Temperature of the fluid within the channels was continuously probed by a thermocouple (IT-24P, Braintree Scientific).

Heat shock application

To apply heat shock we streamed heated Pluronic F127 solution through the second layer channel. The temperature in the device increased linearly in time and stabilized at the required temperature in less than 2 minutes. Heated solution was delivered continuously into the device at a flow rate of 1.5 ml/min. To stop the heat shock the tube of heated Pluronic F127 solution was immersed in water at ambient temperature. Temperature in the channels settled back to 24°C within 4 minutes. For applying a temperature step, we streamed heated Pluronic F127 solution through one of the channels and solution at ambient temperature through the other. For consistency, the hotter channel was always the one closer to the food outlet in the worm layer (guaranteeing that heat is not carried across the worm by the liquid flux).

Operating the device

"Food"

E.coli OP50 was grown overnight in LB media. The overnight culture (OD_{600}=3-4) was spun down and LB was replaced with S-medium to a bacterial final concentration 6 times denser than an equivalent culture at OD_{600}=1.

Worm loading

Worms were loaded into the device as described in Ref. 2. In our hands about 85% of the channels were populated by single worms. Channels occupied by misplaced worms or multiple worms were discarded from imaging and further analysis.

Experimental mode

A reservoir containing ~15ml "food" was placed on a shaker (200rpm) and connected by tubing to the inlet of the microfluidic device. An outlet tube was connected to a syringe pump (New-Era NE-501 OEM) actuated by computer with custom Labview (National-Instruments) that drew liquid from the device at rate of 5 μl/min, thus guaranteeing constant flow of "food". Short pulses of 150 μl/min were applied periodically to clean the device from aggregates and eggs.

Image acquisition

Zeiss Observer Z1 inverted microscope with 10X objective was used for imaging. Images were taken using Hamamatsu Orca II camera with 50ms exposure at 7.8 pixels/10μm resolution. Images were taken every 2 minutes during the heat shock and every 5 minutes before and after.

Data analysis

All data analysis was processed using custom scripts written in MATLAB (Mathworks, MA).

Nuclei counting

Segmentation and quantification of DAF-16 aggregation in nuclei was performed as described in Ref. 2 (**Supporting Methods Fig. 2a-d**).

Profile of DAF-16 accumulation

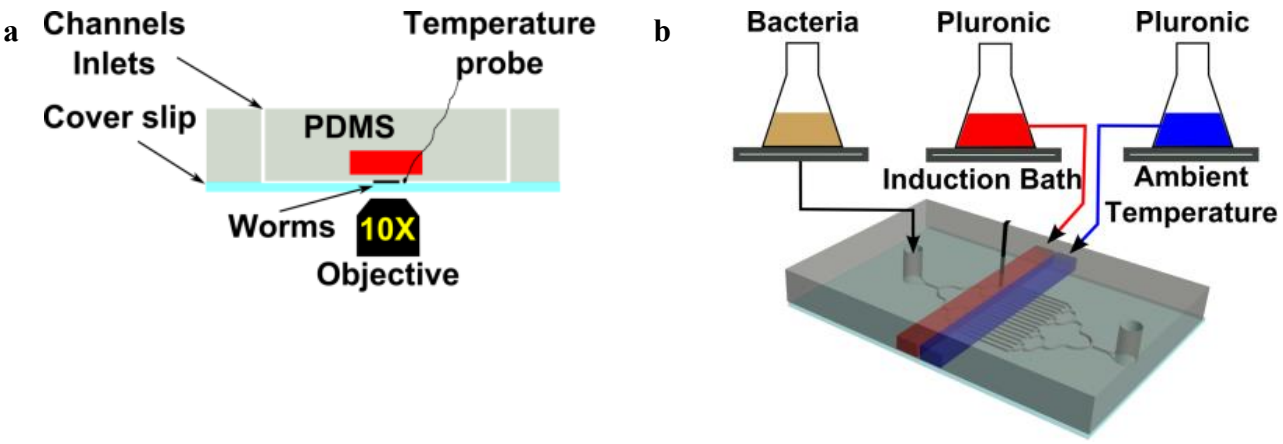
The orientation of each worm in a chamber is maintained during the experiment. However, worms do move slightly back and forth (< 40-50 um over a period of few minutes). Moreover, the length of the worm fluctuates over time due to pharynx and intestinal movements. Though these movements are relatively small compared with the size of the worm, they need to be taken into account during image analysis. In each image (showing a single worm at a given time point) we identified the midline connecting the anterior and posterior end points of the worm, and used to divide the worm into 40 segments of equal width (around 25 um). Each identified nucleus was assigned to the segment that contained its center of mass (**Supporting Methods Fig. 2e**). The total number of activated nuclei in each segment was counted, and is presented in color code the figures.

HSP 16.2 expression analysis

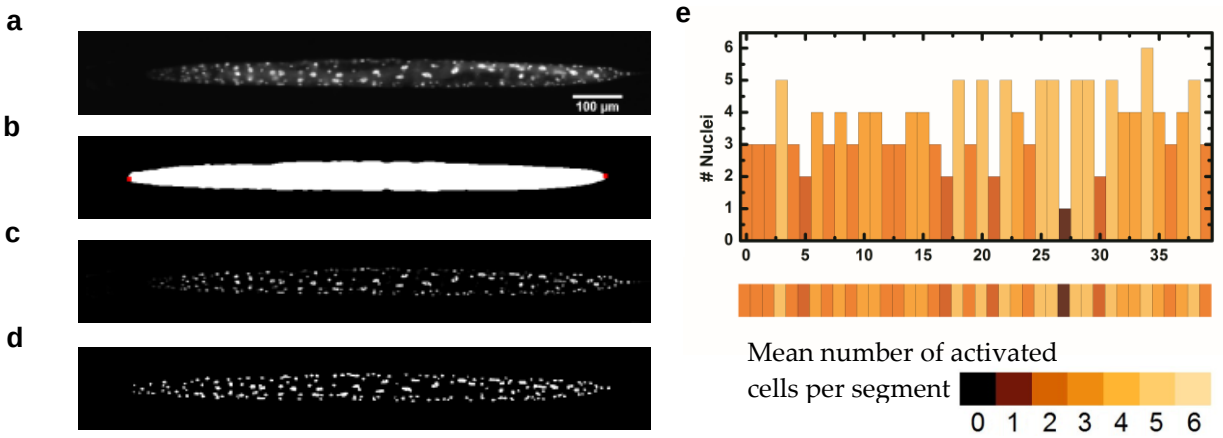
Segmentation of worms to 40 segments was done as above. To quantify the reporter activity we sum the total fluorescence in each segment. Due to the short exposure time we subtract the camera noise, estimated as the mean fluorescence in the other regions of the image. Data was normalized relative to total fluorescence intensity before the heat shock.

References

1. Sulston, J. & Hodgkin, J. in *The Nematode Caenorhabditis Elegans* (Cold Spring Harbor Laboratory Press, 1988).
2. Kopito, R. B. & Levine, E. Durable spatiotemporal surveillance of Caenorhabditis elegans response to environmental cues. *Lab. Chip* **14,** 764–770 (2014).



Supporting Methods Fig. 1 | Microfluidic device. a, Side view. **b,** layout of the experimental setup in experiments where worms are held at two temperatures. Worm bodies are in random orientation perpendicular to the water baths.



Supporting Methods Fig. 2 | Image analysis and data representation. a, Representative raw image. **b,** Detection of worm body by thresholding allows identification of two endpoints (red dots) and definition of the AP axis. **c,** Nuclei showing localized Daf-16::GFP fluorescence are detected by filtering and dynamic thresholding as previously described[2]. **d,** The center of mass of each nucleus is identified. **e,** Worms were divided into 40 equal segments along the anterior-posterior axis, and the average number of identified nuclei in each section was calculated and presented according the specified color scheme. Thus, the average over multiple worms at a given time point corresponds to a single row, and the entire dynamics is represented in the main text as a sequence of such rows.