Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information for

Surface Acoustic Wave Microfluidics for Repetitive and Reversible Temporary Immobilization of *C. elegans*

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Supplementary Videos

Supplementary Video S1

Demonstration of worm immobilization using SAW device. High power SAW (2W, 50% duty cycle) was turned on until worm stops moving (75 seconds). SAW was then switched to low power hold (1W, 50% duty cycle) for 63 seconds. Once SAW was turned off, the worm resumed swimming activity. Video captured at 2 frames per second. Playback at 5x real-time.

Supplementary Video S2

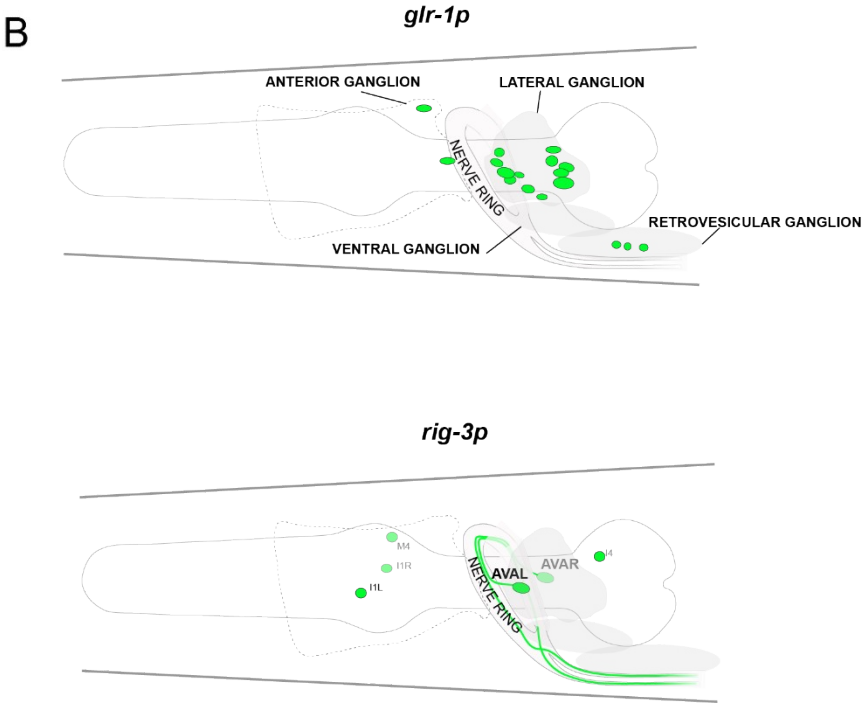
Temperature comparison when immobilizing animals using (A) SAW device and (B) heating plate. Temperature was recorded at the exact moment when worm stopped moving. SAW device showed a temperature of 28.1 °C, while heating plate showed a temperature of 34.6 °C. Videos captured at 4 frames per second. Playback at 2x real-time.

Supplementary Figure S1 Illustration and list of neurons expressing the *C. elegans* ionotropic glutamate receptor homologue GLR-1 tagged with GFP under either native *glr-1p* or AVA specific *rig-3p* promoters. (A) Table describing neuronal expression pattern and the ventral nerve cord neurons for each promoter. (B) Illustrations of neurons expressing GLR-1 tagged with GFP in the head of *C. elegans* animals under the control of the *glr-1p* or *rig-3p* promoters.

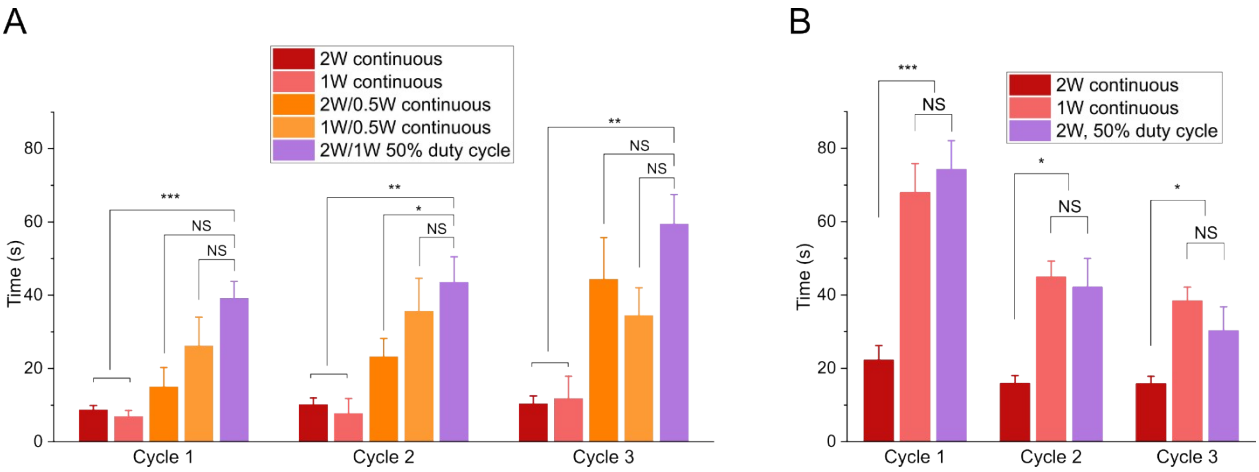
A

Promoter	Neuronal expression pattern	Ventral nerve cord neurons
glr-1p	AIB, AVA, AVB, AVD, AVE, AVG, AVJ,DVC, PVC, PVQ, RIM,RIS, RIG, RMD,RME,SMD, SMDD, SMDV	AVA, AVB, AVD, AVE, AVG, AVJ, DVC, PVC, PVQ, SMDV
rig-3p	AVA, I1, I4, M4, NSM, inconsistent in PVT	AVA

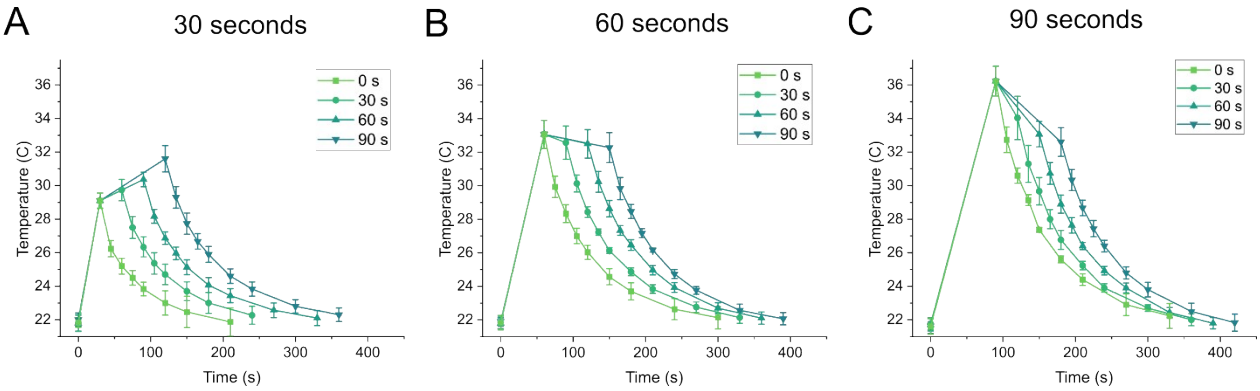
B



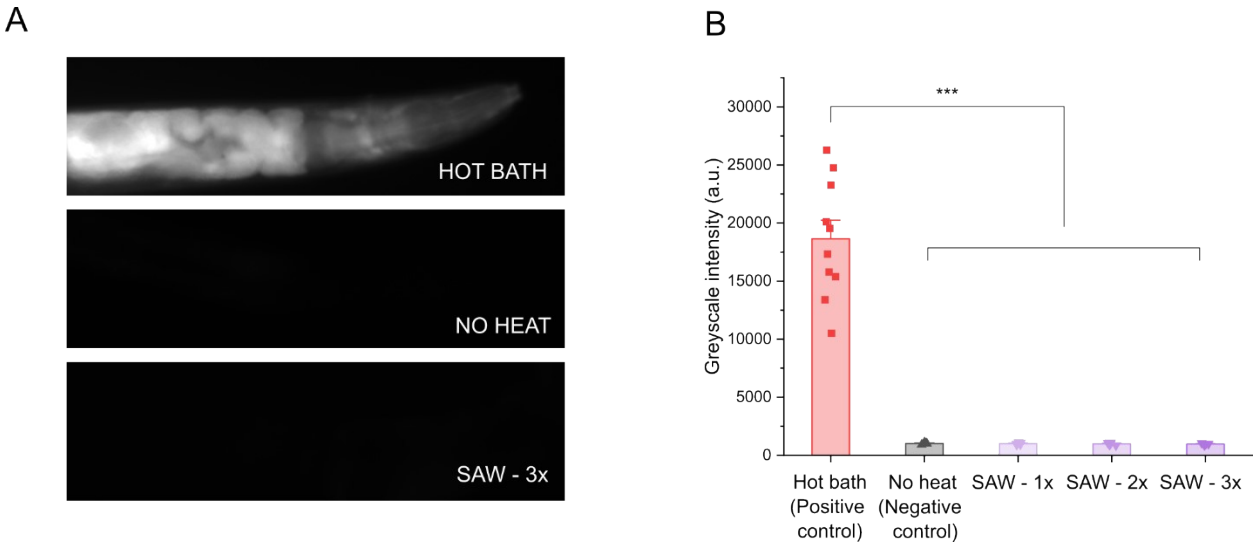
Supplementary Figure S2 Characterization of worm immobilization and recovery using SAW device. (A) Time required to immobilize animals using different SAW power levels and duty cycles. (B) Total immobilization time after SAW without (red) or with (orange, magenta) a low power hold step. All conditions led to animal recovery after SAW and immobilization, Data are mean ± s.e.m. For each power level, at least 10 animals were tested for 3 cycles each. *P< 0.05, **P<0.01, ***P< 0.001, two-sided, unpaired t-test.



Supplementary Figure S3 Temperature characterization of device showing initial high-power SAW (2 W-50% duty cycle for (A) 30 seconds, (B) 60 seconds, and (C) 90 seconds and corresponding low-power SAW (1 W-50% duty cycle) hold times. Device cooling after SAW was turned off was also tracked. Data are mean ± s.e.m. Three individual experiments were performed for each temperature curve.



Supplementary Figure S4 Heat shock characterization. (A) Wide-field fluorescent images (20x objective, 500 ms exposure) of representative *ST66* animals 1 hour after (i) positive control – 1-hour hot bath at 32° C, (ii) negative control – no heat applied, and (iii) 3x SAW immobilization cycles. (B) Greyscale intensity after measurements comparing positive and negative controls with 1, 2, and 3 SAW immobilization cycles. Data are mean ± s.e.m. At least 10 individual animals were imaged for positive control, and 5 individual animals were imaged for all other experiments. ***P< 0.001, two-sided, unpaired t-test.



Supplementary Figure S5 SAW induced worm deformation and recovery. A) Length and (B) width percent change measured during one SAW immobilization cycle. Measurements were taken during the change from free-swimming to immobilized state, and from immobilized to free-swimming state. (C) Length and (D) width percent change measured once each day for 48 hours. Data are mean ± s.e.m. At least 19 animals were measured for A and B, and at least 7 animals were measured for C and D. ***P< 0.001, **P< 0.01, *P< 0.05, two-sided, unpaired t-test.