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

Supplementary Information

Microfluidic approach to correlate *C. elegans* neuronal functional aging and underlying changes of gene expression in mechanosensation

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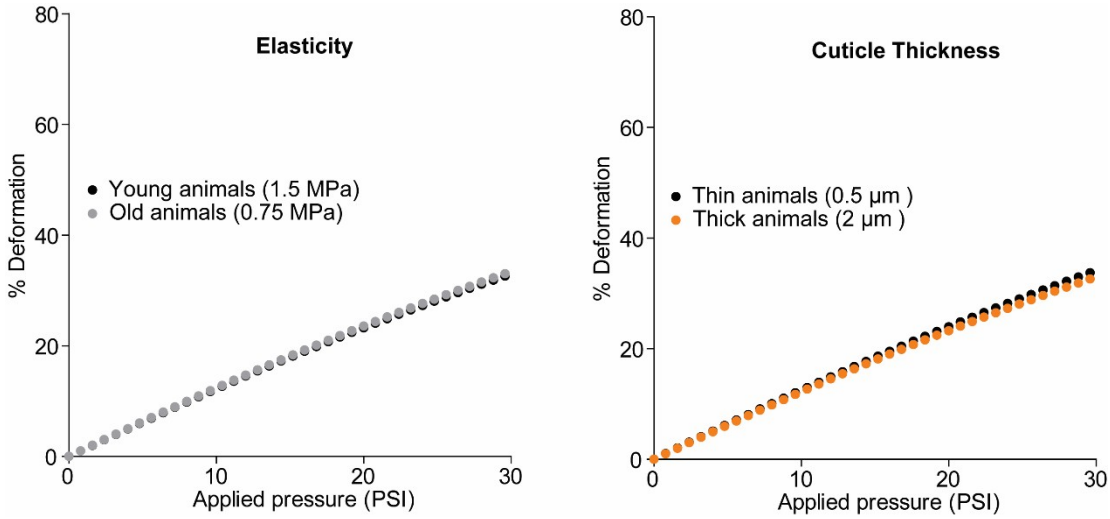
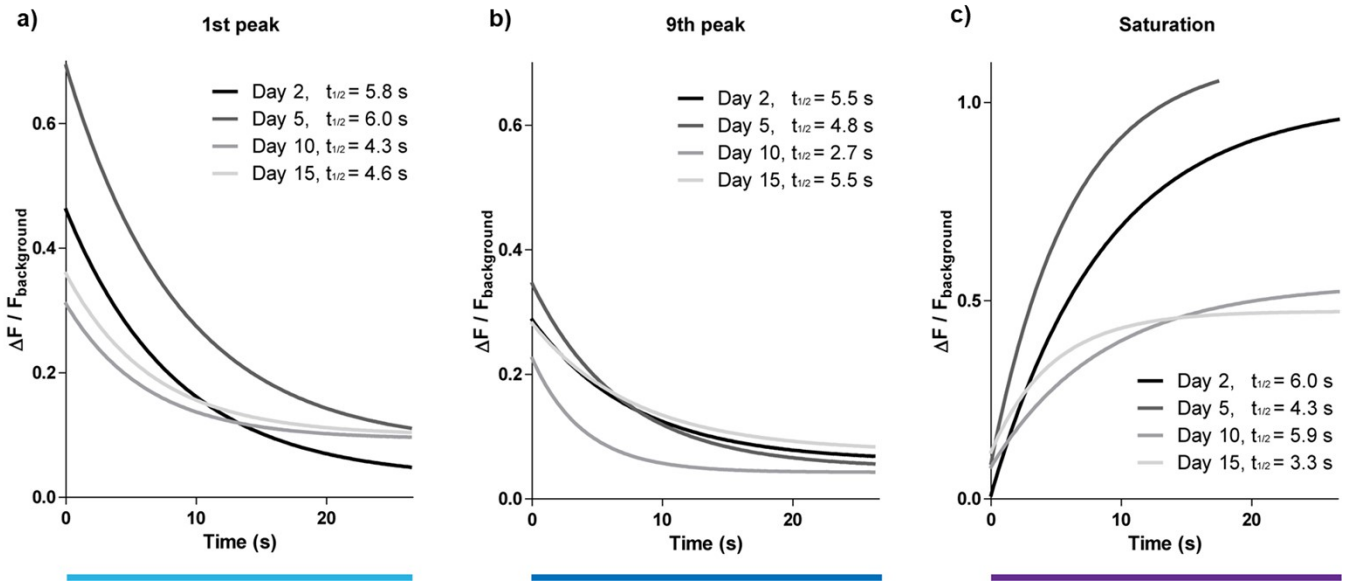
Supplemental Figure 1



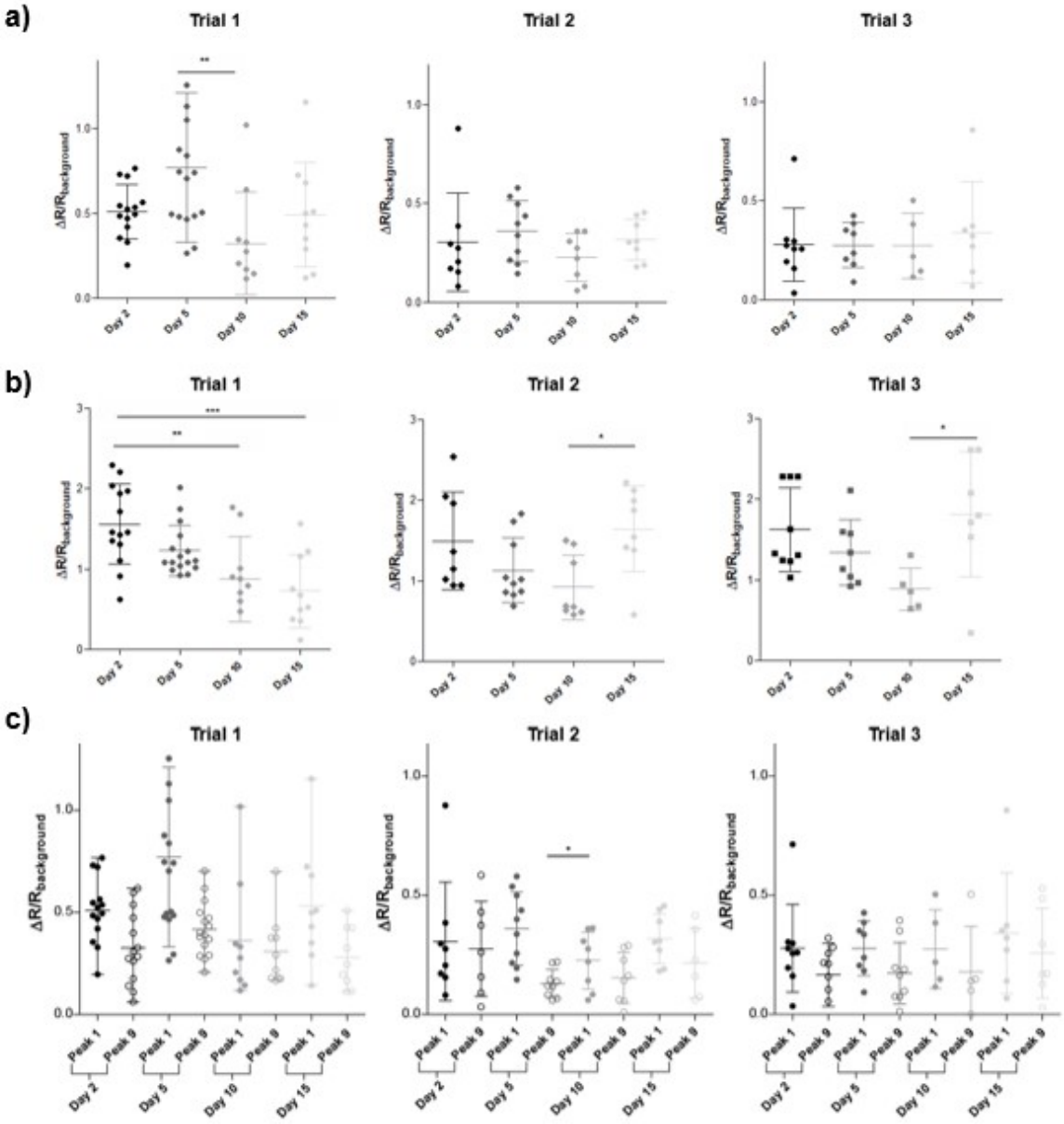
Supplemental Figure 1. Changes in worm properties are negligible for device operation. The effects of the animals' natural changing properties (elasticity decrease and cuticle thickness increase with age) are very slight. When compared to the batch-to-batch variations of the properties of the PDMS-based devices, these natural, biological changes are negligible.

Supplemental Figure 2



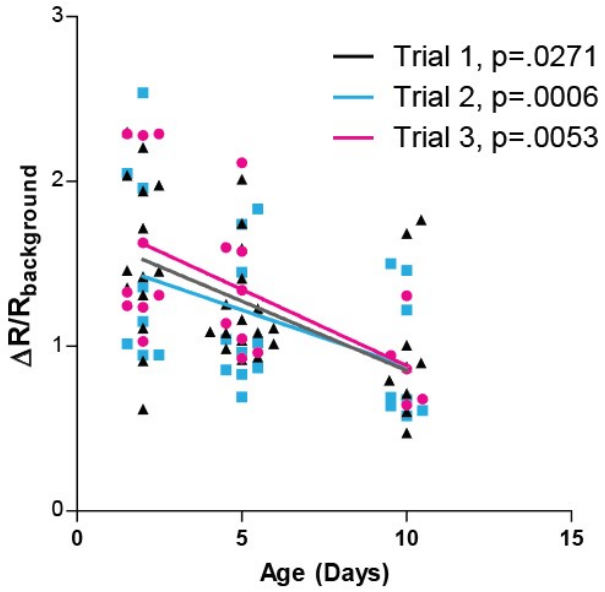
Supplemental Figure 2. Age-related changes in dynamics of neuronal function. a,b) Following the stimulus, there is a recovery period to baseline. To quantify the changes in dynamics, we fit a one-phase decay curve to the data. a) Older animals had a shorter half-life, indicating that the time to return to baseline is shorter perhaps due to the smaller initial peak. This trend was not seen after the 9th stimulus, indicating perhaps habituation affects the curves' dynamics. c) One-phase association curve fit to the final long pulse stimulus data. Day 15 had the shortest half-life, suggesting it has the shortest peak but also fastest response. In contrast, Day 10 reached a similar peak to Day 15 but had a slower increase, perhaps due to animals with unhealthy neuronal function in the population.

Supplemental Figure 3



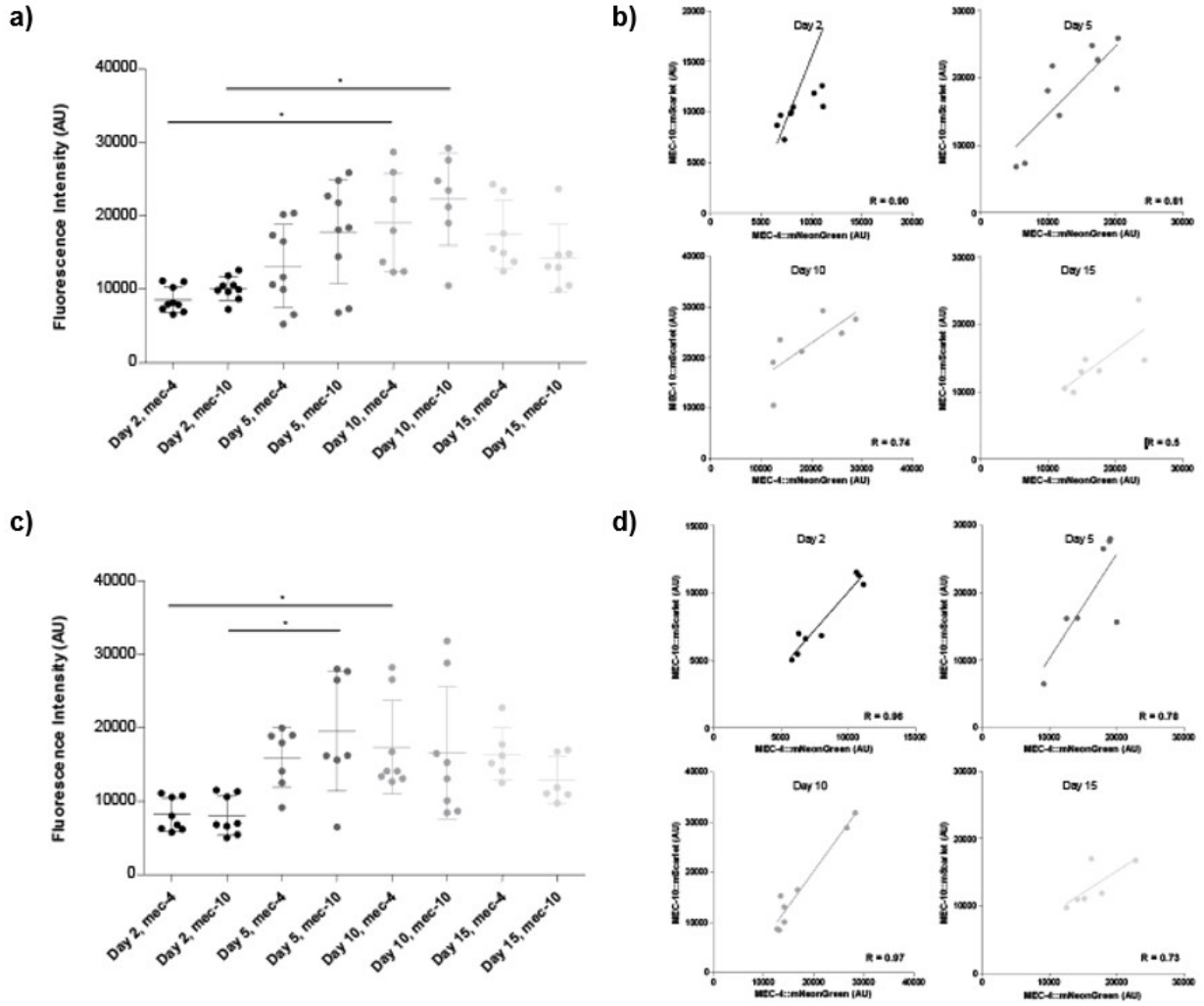
Supplemental Figure 3. Replicate trials produce similar trends in age-related functional change. a) The magnitude peak height of individual for each age group are compared across three trials. There is no statistically significant difference between any age group except for Day 5 and Day 10 for Trial 1. b) The trial-to-trial variation for the long stimulus pulse are shown. There is a statistically significant difference between Day 2 and Days 10 and 15 for Trial 1, whereas there is a statistically significant difference between Day 10 and Day 15 for Trials 2 and 3. c) The difference between the first and ninth response to the short stimulus pulse are shown for all trials. In all but one case, Day 5 of Trial 2, there is not a statistically significant difference between the first and ninth response. The Kruskal-Wallis test was performed, followed by Dunn's multiple comparison test (* $P < 0.05$, ** $P < 0.01$, *** $P < .001$).

Supplemental Figure 4



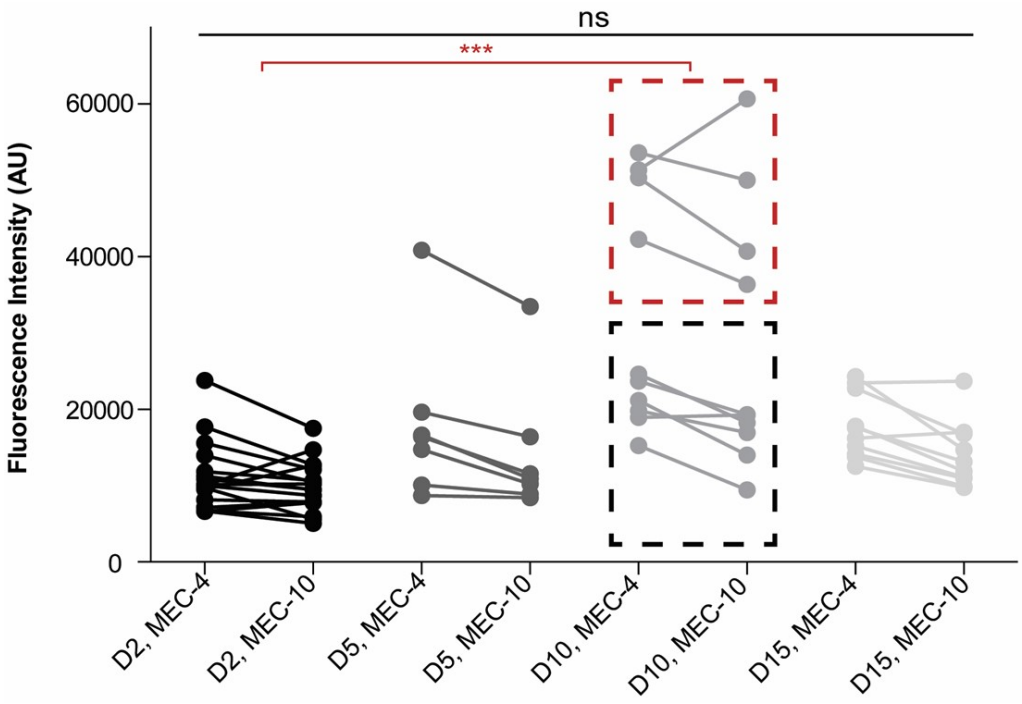
Supplemental Figure 4. Linear regression reveals declining trend in response to long stimulus between Days 2 and 10. For each trial, linear regression is performed and a significantly non-zero slope, with p-values included in the legend, is found. The slope is seen to be similar between trials: the slope within 95% confidence, for Trials 1, 2, and 3 is -.128 to -.0084, -.130 to -.028 and -.154 to -.031, respectively.

Supplemental Figure 5



Supplemental Figure 5. Replicate trials produce similar trends in gene expression. a) Trial 2 MEC-4 and MEC-10 expression for each age group are compared. There is a statistically significant increase in expression between Day 2 and Day 10 for both MEC-4 and MEC-10, similar to what is shown for Trial 1 in Figure 5. b) The correlation between MEC-4 an MEC-10 expression levels for Trial 2 are shown. Results are similar to what is seen for Trial 1 in Figure 5. c) Trial 3 expression levels and d) correlation between MEC-4 and MEC-10 are shown for each age group. There is a significant increase in expression between Day 2 and Day 10 for MEC-4 and Day 2 and Day 5 for MEC-10. Unlike the other two trials, there is not a statistically significant difference between Day 2 and Day 10 for MEC-10, demonstrating the impact of low sample size and high individual variance. The Kruskal-Wallis test was performed, followed by Dunn's multiple comparison test (* $P < 0.05$).

Supplemental Figure 6



Supplemental Figure 6. Individual animals' MEC-4::mNeonGreen and MEC-10::mScarlet-I expressions are strongly correlated and the high-expressing population in Day 10 is responsible for the differences between the ages. Each animal's MEC-4 and MEC-10 expression levels were related (line between the points). While there is an increase at Day 10, only the high-expressing animals of the population are responsible for the significant difference between other ages (red box). There is perhaps a bimodal distribution of animals at Day 10, where the high expressing population is not present at Day 15.