7 Supplementary Figures

Fig. S1: The simple fabrication process for our device uses a standard soft-lithography process with only one lithography step. We use a 2 μm layer of SU-8 to improve the adhesion of small structures to the wafer. Here we show a cross-section of the final structure (not to scale).

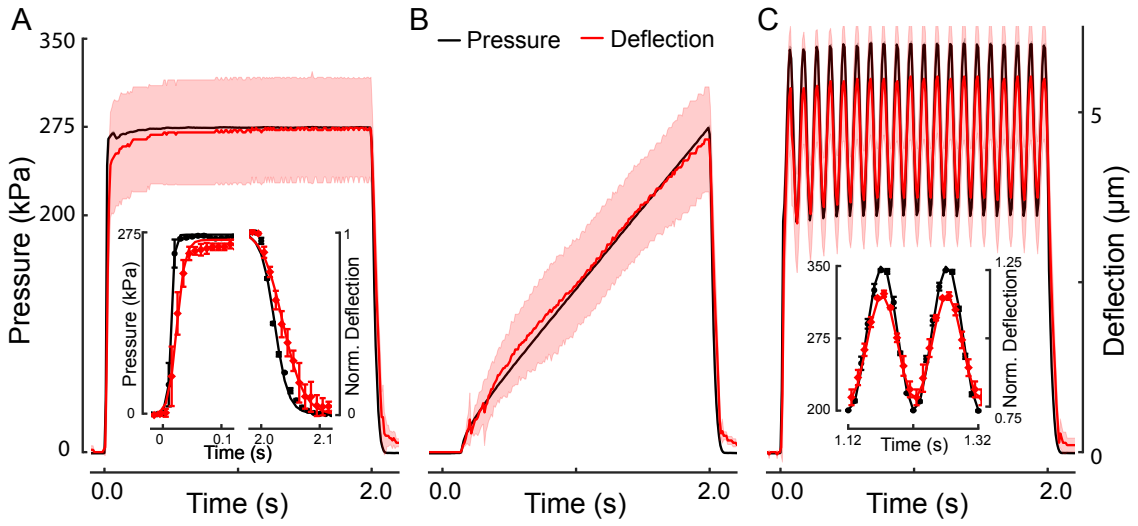


Fig. S2: Temporal characterization of the diaphragm deflection for the step, ramp and buzz stimulus. **A:** Average pressure (left axis, black) and diaphragm deflection (right axis, red) vs time of the step stimulus for three independent measurements (N=3 actuators). Shaded area represents the SD of the three traces. SD on pressure is too small to be visible. Left inset shows a higher magnification of the pressure and deflection increase. A sigmoidal fit (solid line) was used to determine the 10% and 90% rise time of the pressure and the resulting diaphragm deflection. We used these values to calculate the 10-90 rise time, which describes the time it takes for a step to go from 10% of the final value to 90% of the final value. The average time lag between the two time series was determined to be 20±3ms (mean±SD, N=3). Right inset shows a higher magnification of the pressure and normalized deflection decrease. Deflections in the inset were normalized to the max deflection. The time-lag between the pressure release and diaphragm relaxation was determined to be 30±16ms (mean±SD). **B:** Average pressure (black) and diaphragm deflection (red) vs time of the ramp stimulus (N = 3 actuators). Strain rate of the ramp was measured to match 2.4±0.5 µm/s (mean±SD). **C:** Plot showing average input pressure (black line) and resulting deflection (red line) of the diaphragm of the buzz stimulus. The insets at the buzz stimulus show the average pressure and the average, normalized deflection each fitted with a sine function (each N = 3). The lag time between the two timeseries ($\approx$ 1 ms) as determined from the fit was well below the sampling period (10 ms) and thus below the resolution limit of our imaging procedure.

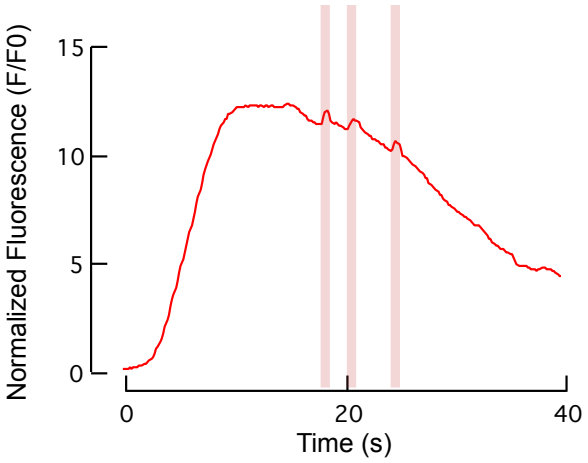


Fig. S3: Endogenous blue light activation of TRNs masks mechanical activation

LITE-1-induced calcium transients after illuminating worms with blue light in control worms masks mechanical activation of ALM by the buzz stimulus (red shaded areas).

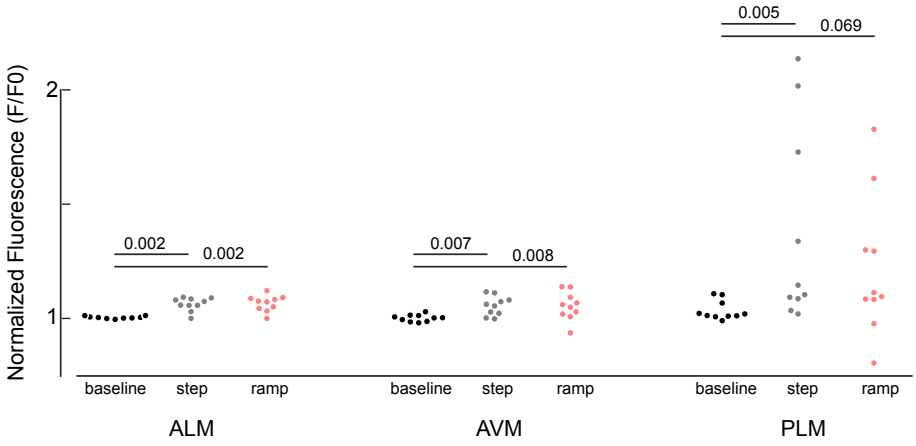


Fig. S4: High pressure activates TRNs

The maximum pressure of 450 kPa induces calcium transients in all TRNs after stimulation with a step and a ramp. The p-values were derived from Wilcoxon rank sum test (N=10).

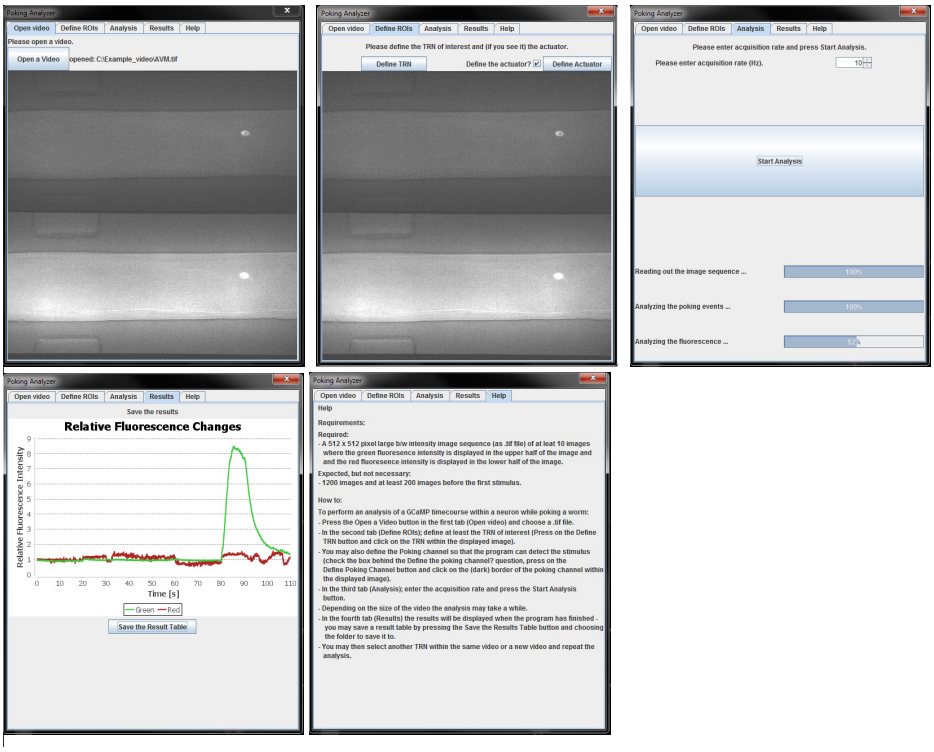


Fig. S5: Screen shots of Worm Poker

The accompanying software is freely available under `https://github.com/HFehlauer/Poking-Analyzer/blob/master/pokinganalyzer_`

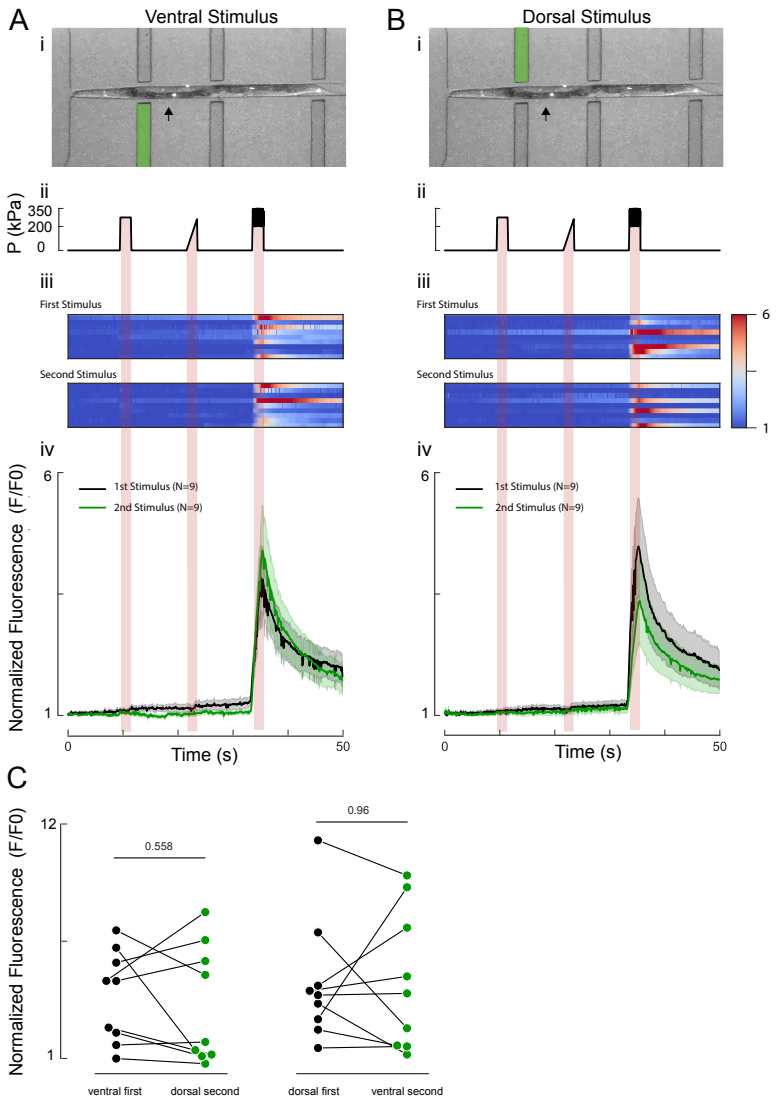
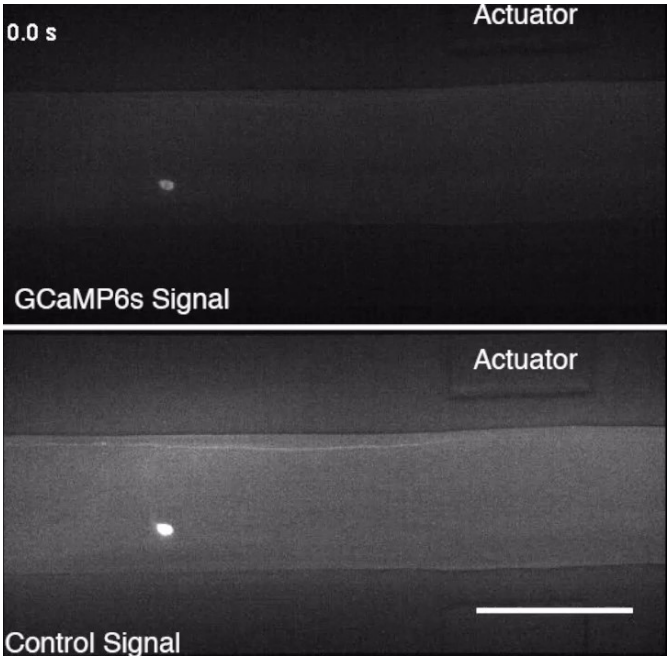


Fig. S6: Calcium dynamics of the AVM neuron as a response to ventral vs. dorsal stimulation

Response dynamics of AVM when stimulated from the ventral (**A**) or dorsal (**B**) side. Worms were either first stimulated from the ventral side followed by a stimulation from the dorsal side or vice versa. **(i)** Overlay of a brightfield and a fluorescence image showing a worm in the microfluidic chip. The actuator used for stimulation of the AVM neuron from ventral or dorsal side are marked in green, arrows point toward the cell body of the AVM neuron. **(ii)** Stimulus protocol including 2 second diaphragm excitation representing a 275-kPa step, a 275-kPa ramp and a sine (75 kPa; 10 Hz) superimposed with a 275-kPa step (buzz). **(iii)** Multiple false color-coded normalized fluorescence intensity traces (F/F_0) during the mechanical stimulation (shown in (i)). Traces beneath first stimulus show calcium responses after stimulation from the according side, traces beneath second stimulus show calcium responses when the neuron was stimulated from the respective side after being stimulated from the opposite side. **(iv)** Average fluorescence intensity (F/F_0) of the traces shown in (ii) for first stimuli and second stimuli (mean $\pm$ SEM as shaded area, N=9). **C**: Maximum amplitudes of the calcium signal following the buzz stimulus when worms where first stimulated from the ventral side followed by stimulation from the dorsal side or vice versa. Responses from the same neuron are connected by a black line. p-values derived from a paired t-test are shown above the black bar.



Movie S1: First frame of the supplementary, representative movie of AVM neurons subjected to the stimulation protocol in Fig. 5 (scale bar 50 μm)