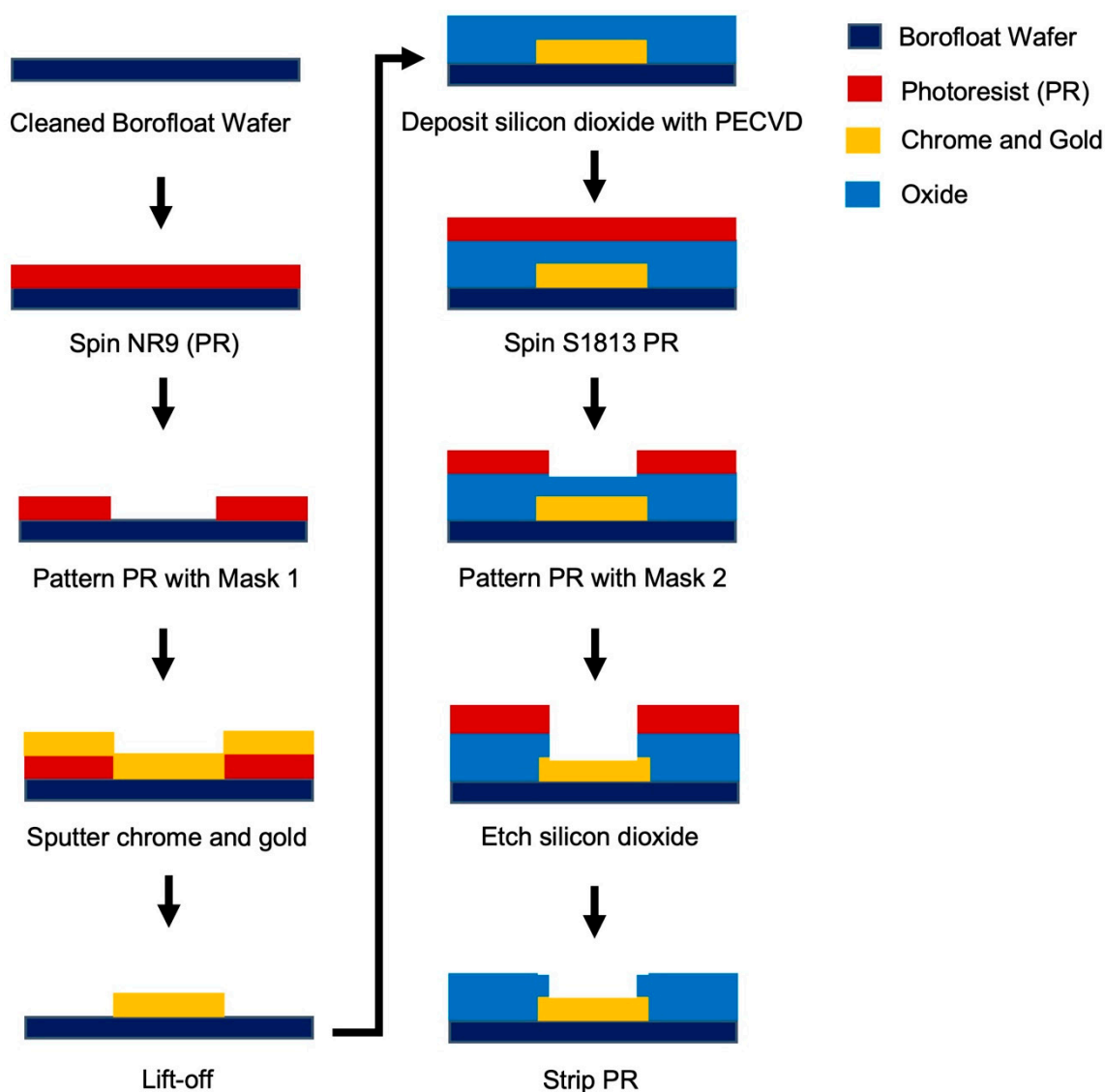


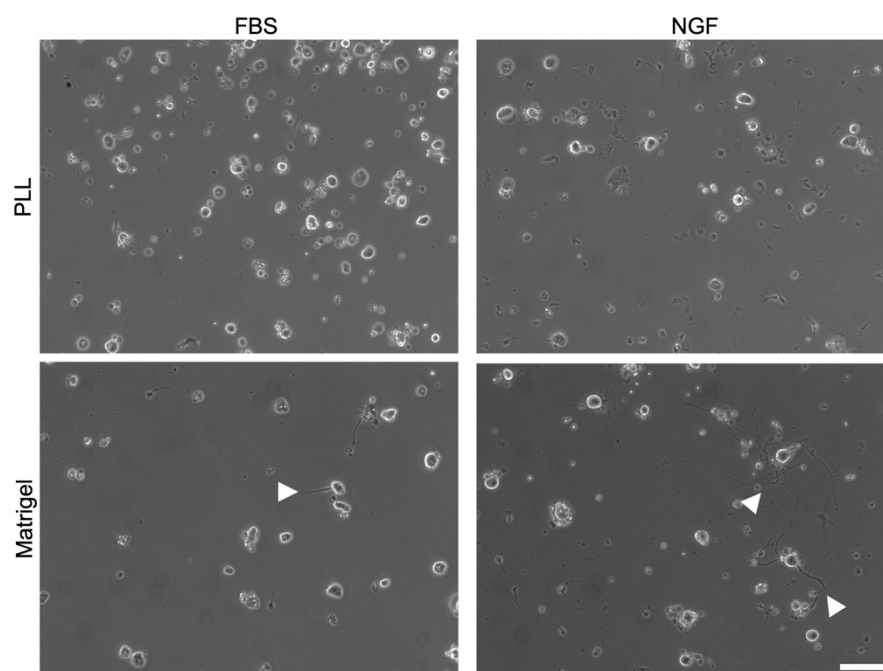
Supporting Information

# Cultured Vagal Afferent Neurons as Sensors for Intestinal Effector Molecules

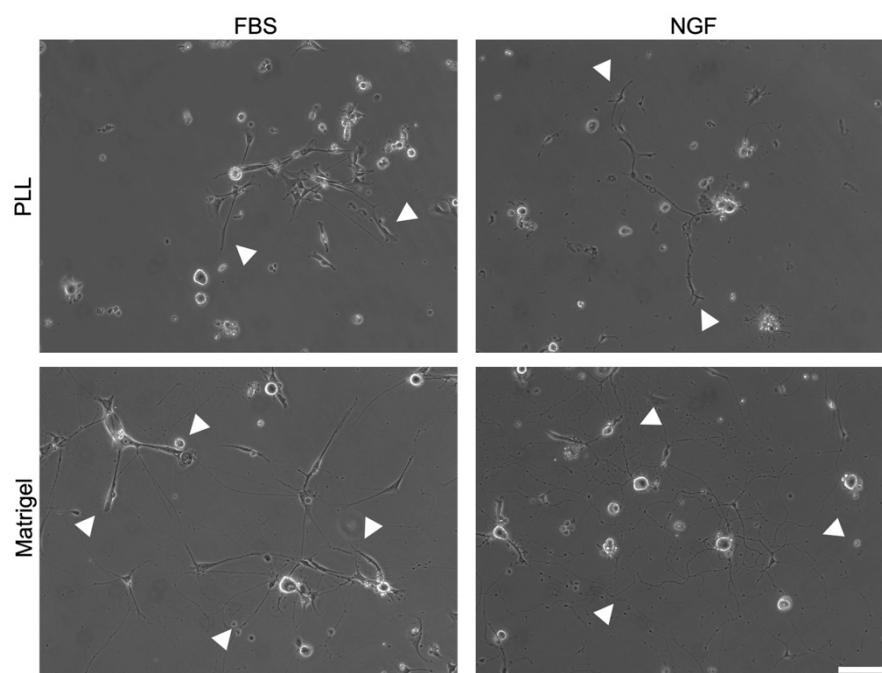
Gregory Girardi, Danielle Zumpano, Noah Goshi, Helen Raybould and Erkin Seker \*



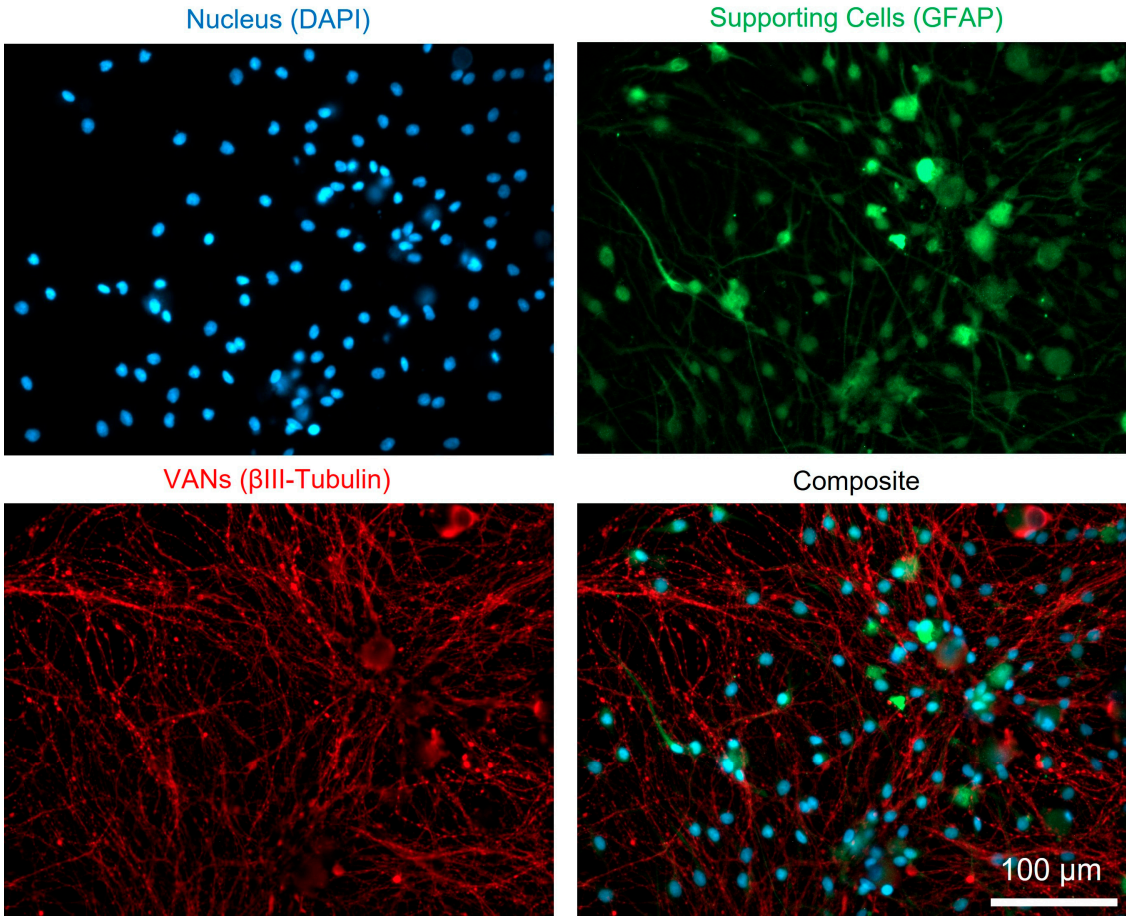
**Figure S1. Schematic of microelectrode array fabrication steps.** Cleanroom techniques including thin-film metal deposition and photolithography were used to fabricate custom microelectrode arrays. Briefly, gold with a chrome adhesion layer was deposited onto a glass wafer to pattern the electrodes (mask 1), silicon oxide was deposited to insulate the electrodes, and opened only above the recording regions (mask 2) with a buffered oxide etch (BOE).



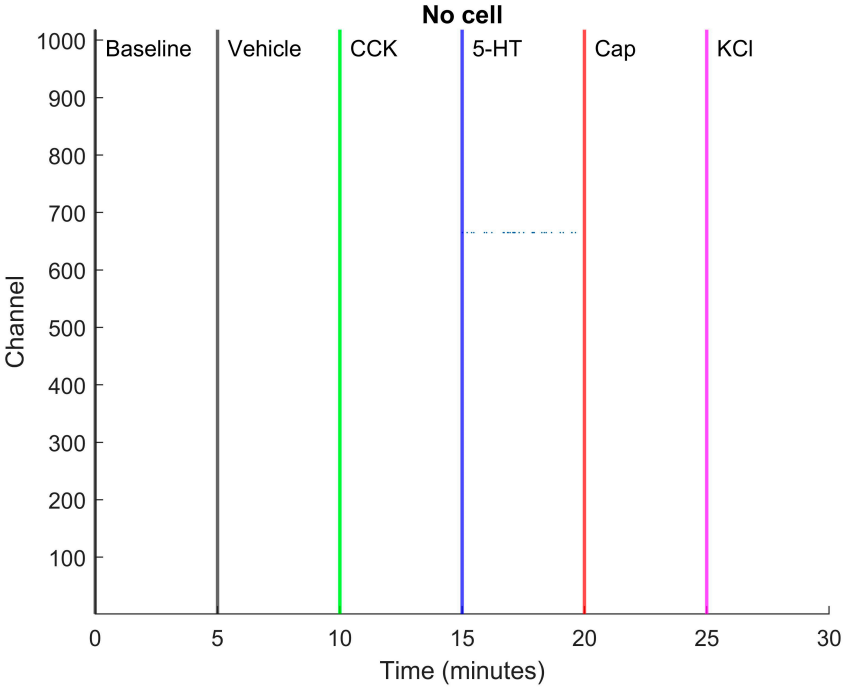
**Figure S2. Day 1 VAN culture.** Media comparison of serum-based media (DMEM + FBS) to growth factor-based (DMEM/F12 + NGF). White triangles indicate neurite outgrowth. Scale bar 100  $\mu$ m.



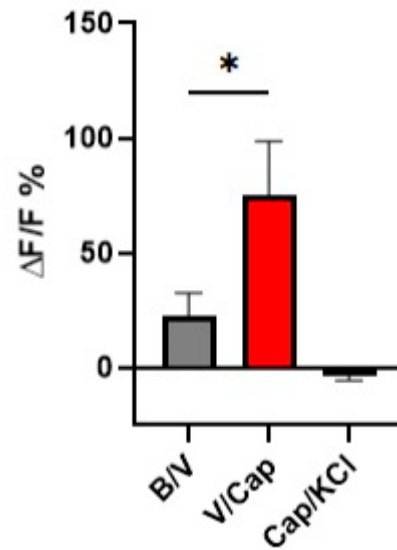
**Figure S3. Day 3 VAN culture.** Media comparison of serum-based media (DMEM + FBS) to growth factor-based (DMEM/F12 + NGF). White triangles indicate neurite outgrowth. Scale bar 100  $\mu$ m.



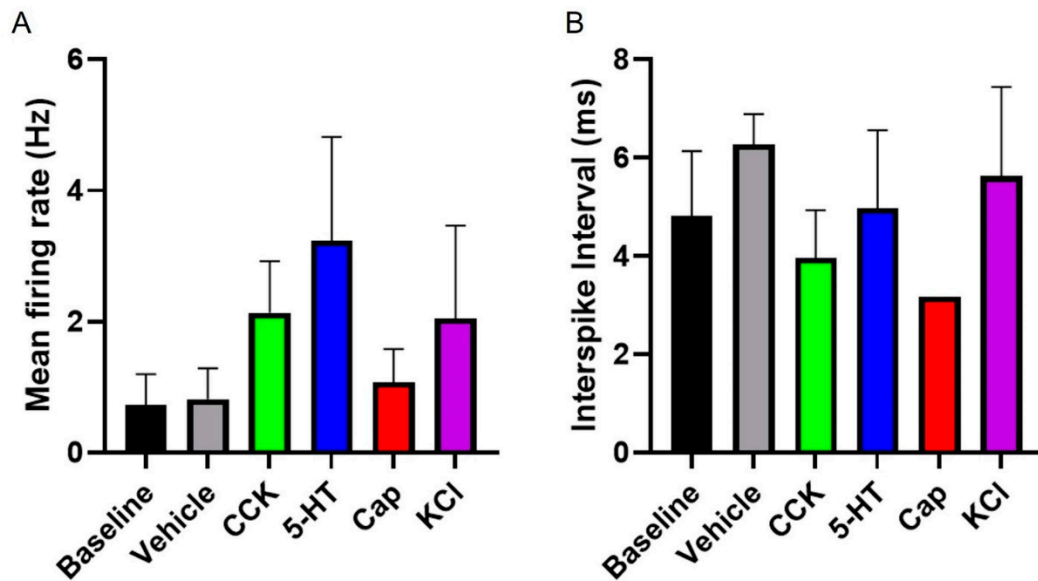
**Figure S4. Day 7 VAN culture.** Immunostained (DAPI – nuclei, GFAP – supporting cell,  $\beta$ T-III – neuron) VAN culture on day in vitro 7 highlighting that at longer culture durations, non-neuronal supporting and proliferative cells (GFAP+) are present. Scale bar 100  $\mu$ m.



**Figure S5. Raster plot for HD-MEA chip with no cells.** A chip containing no cells shows negligible signal. Allowing us to trust that our signals recorded from chips with cells are real cellular signals.



**Figure S6. Intracellular calcium response after Cap exposure.** Elevated extracellular potassium (20 mM) after Cap (1  $\mu$ M) exposure does not change  $\Delta F/F$  %. Highlighting that these neurons became desensitized from Cap. \*  $P \leq 0.05$ .



**Figure S7. Averaged extracellular recordings firing characteristics.** (A) Mean firing rate (Hz) under each effector molecule group, highlighting the increase in firing rate from CCK, 5-HT, and KCl, while also showcasing the drop-in mean firing rate under Cap. (B) Interspike interval (ms) under each effector molecule group showcasing the decrease in interspike interval under Cap.