

Introduction

The development of new pain therapeutics has been hampered by the lack of reliable preclinical models. To address this challenge we have established a novel preclinical discovery strategy, which relies on the *ex vivo* interrogation of dorsal root sensory neurons isolated from human organ donors. Here, we present a model for studying the efficacy of new compounds to treat different types of pain. By adding proinflammatory or neuropathogenic agents to culture conditions, we investigated how the physiological properties of cells are altered in our pain models.

Methods

Cell isolation & culture. Human dorsal root ganglia (hDRGs) were collected within 2 hours post-mortem and transferred to AnaBios using proprietary cold preservation solution. Cells were enzymatically dissociated, plated on PDL-coated glass coverslips, and cultured in DMEM-F12 in the presence of 10 ng/mL NGF and 10 ng/mL GDNF, at 37°C with 5% CO₂.

Fluorescence calcium imaging. hDRG neurons were loaded with the calcium sensor Fluo-8-AM and imaged at 100 Hz with a pcoEDGE sCMOS camera (PCO) mounted on an inverted microscope.

Electric field stimulation (EFS). A pair of carbon fiber rods was used as stimulating electrodes; trains of biphasic pulses with 10 ms duration and a frequency of 5 Hz were used to elicit calcium transients. Stimulus intensity was set at 7.5 V/cm to 15 V/cm.

Electrophysiology. Individual hDRG neurons were stimulated at frequencies of 0.1, 1, 3 and 10 Hz with trains of 120 depolarization pulses (20 ms in duration) delivered at 150% of rheobase. hDRGs were treated with 500 nM Prostaglandin E₂ (PGE₂) and 50 nM bradykinin (BK) for 72 hours to model inflammation. To model chemotherapy-induced neuropathic pain, hDRGs were treated with 50 µM Oxaliplatin, or 100 nM Paclitaxel for 24 hours.

Results

Fluo-8-AM Calcium Imaging of hDRGs

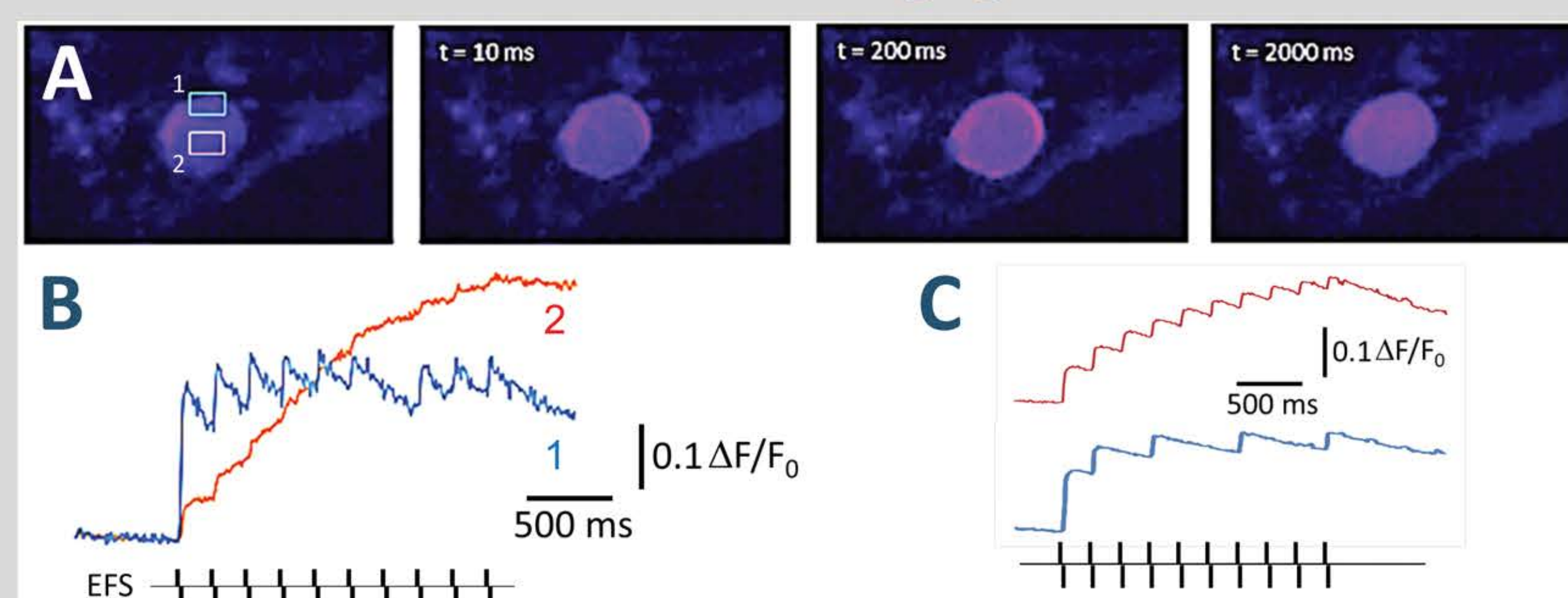


Figure 1. Intracellular calcium transients elicited by EFS in human DRG neurons. **A.** Time-lapse images from a recording of an hDRG neuron showing intracellular Ca²⁺ dynamics evoked by EFS. **B.** Ca²⁺ transients at submembrane location (ROI/Trace #1) or at a cytoplasmic location (ROI/Trace #2) for the cell shown in **A.** **C.** Whole-cell Ca²⁺ transients measured in 2 hDRG neurons. *Red trace:* example of a neuron that was able to follow the entire stimulus train. *Blue trace:* neuron exhibiting failures while following the stimulus train.

Inflammation Increases the Percentage of TTX-resistant Cells

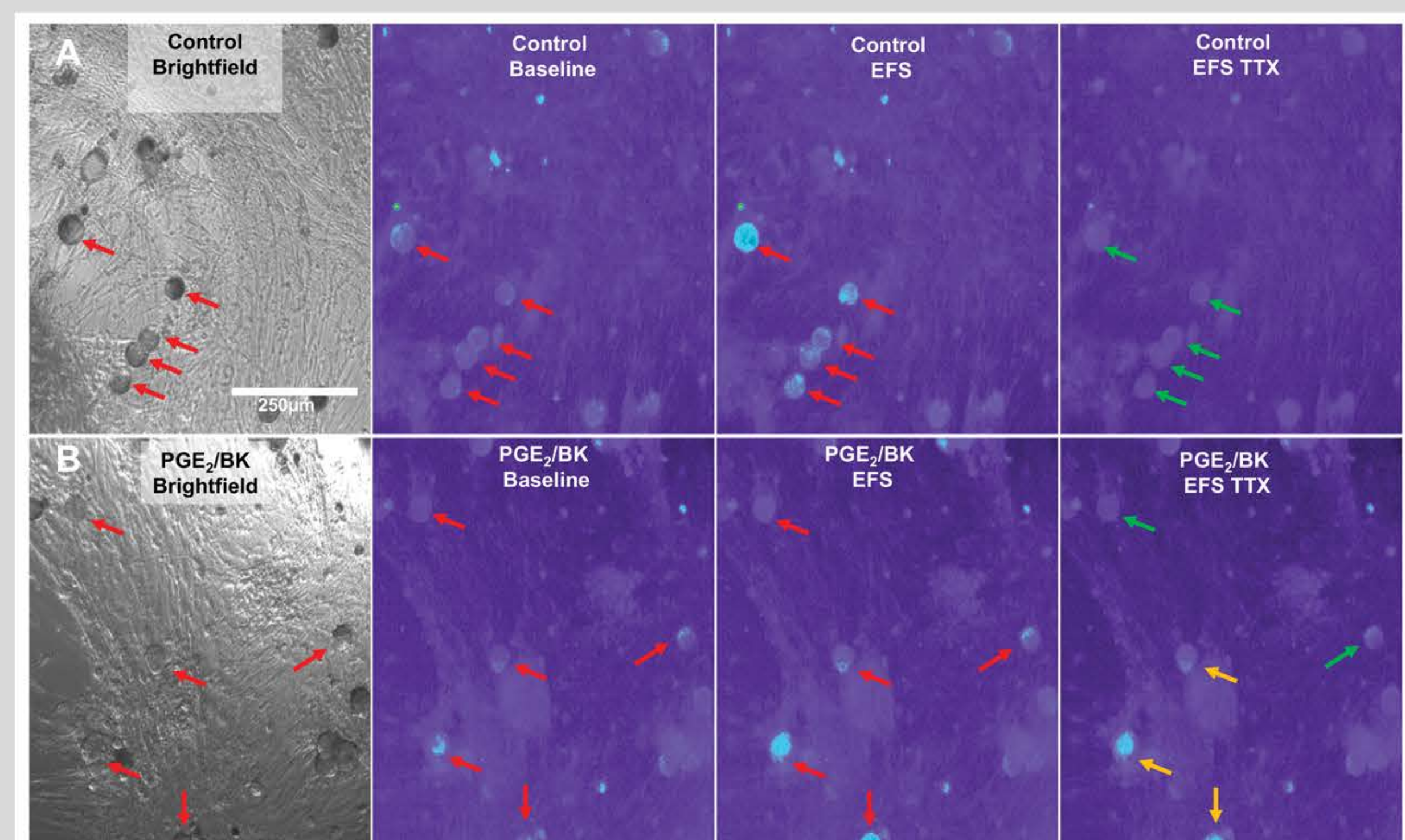


Figure 2. EFS recordings of human DRG neurons under control conditions and after treatment with PGE₂/BK. **A.** Example of EFS recordings under control conditions. **B.** Example of EFS-induced responses following a 2 hour treatment with 1µM PGE₂ and 100nM BK: inflammation reduced the number of cells that exhibited sensitivity to TTX compared to control. Green arrows: TTX sensitive (TTX-S); yellow arrows: TTX resistant (TTX-R).

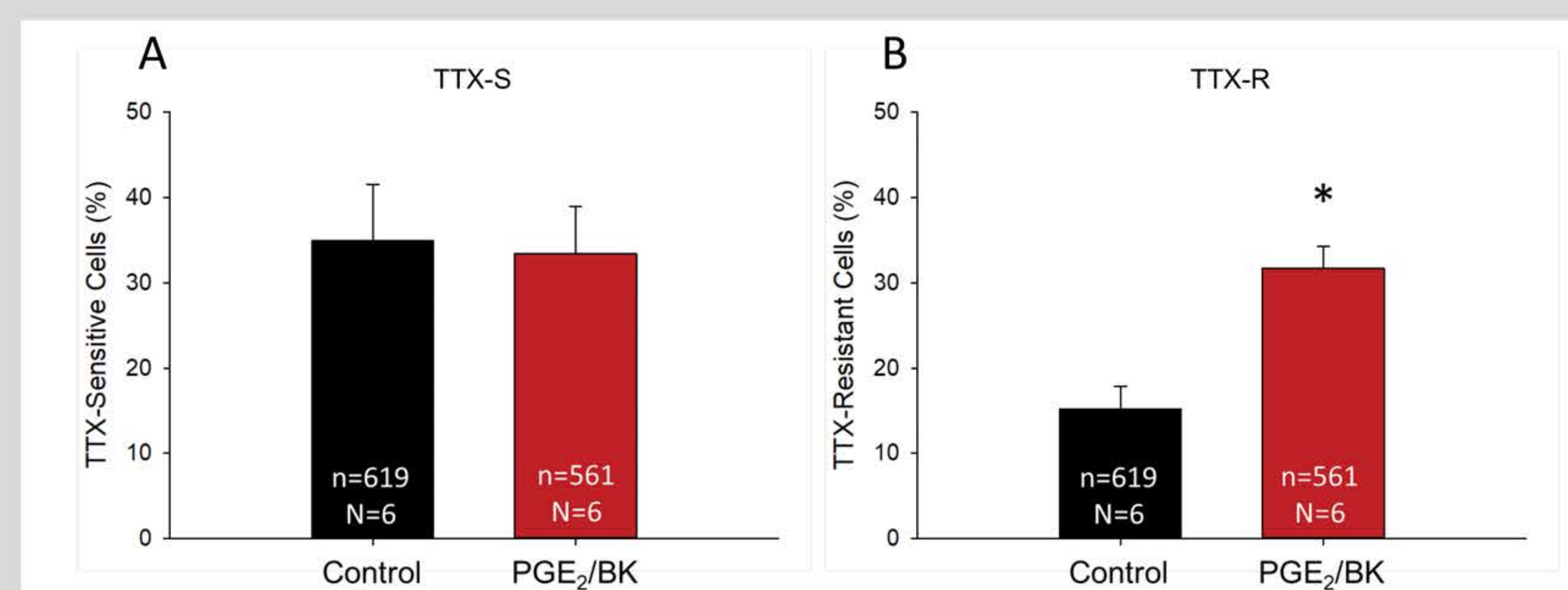


Figure 3. Effect of PGE₂/BK on TTX sensitivity of hDRG neuron on EFS-induced responses. **A.** The percentage of TTX-S neurons was unchanged by 2 hours of PGE₂/BK treatment. **B.** Treatment with PGE₂/BK significantly increased the percentage of TTX-R neurons. (n = number of cells; N = number of donors; *p<0.05).

Inflammation Changes the Functional Phenotype of hDRGs

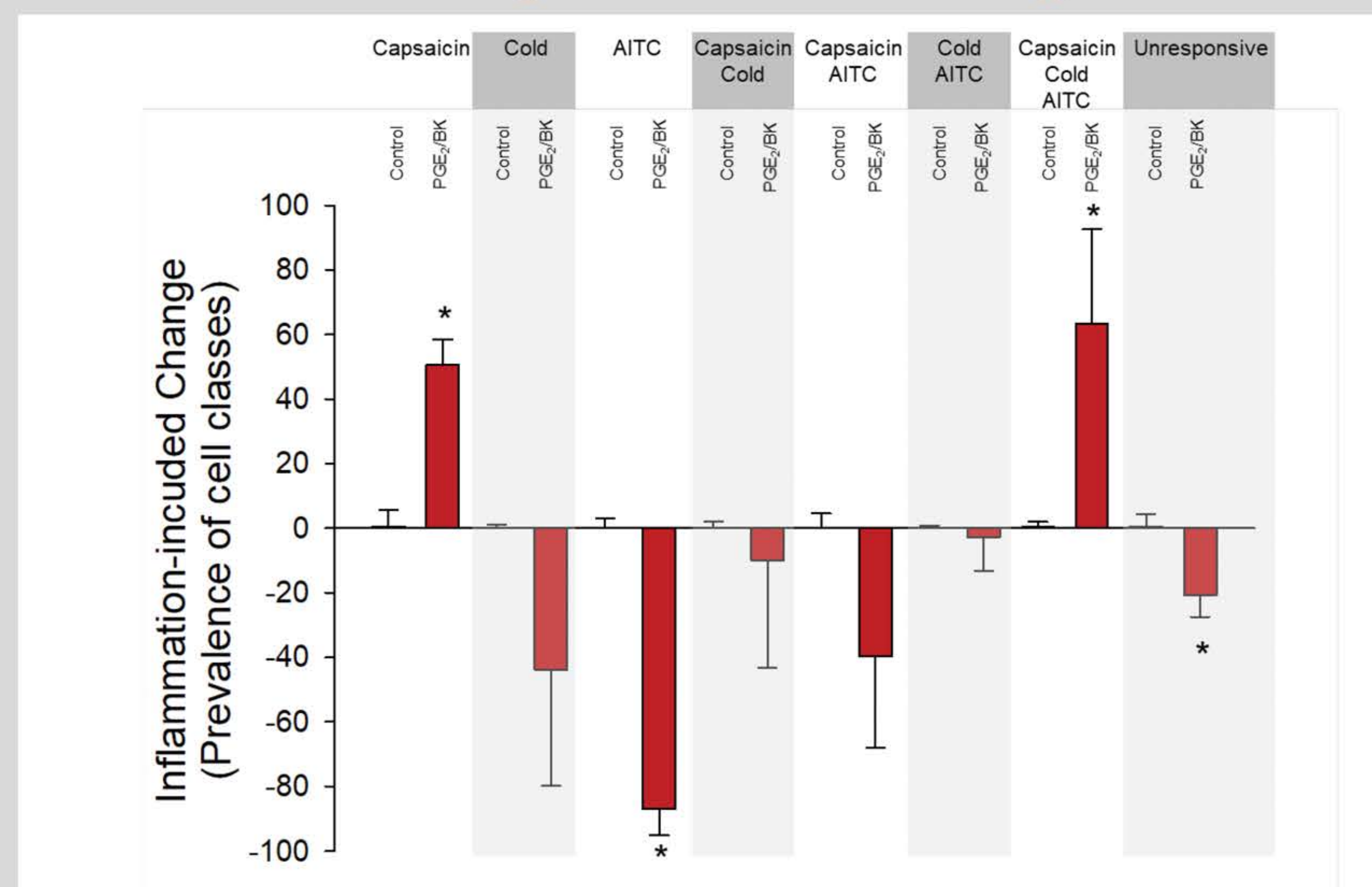


Figure 4. Effect of PGE₂/BK on hDRG responses to capsaicin, AITC and cold buffer. hDRG neurons were incubated for 2 hrs. in PGE₂/BK. Cells were sequentially challenged with 100 nM Capsaicin, 50 µM AITC, and ~13°C cold buffer. The figure shows changes in the prevalence of cells responsive to single agonist application or responsive to multiple agonists (control: n = 306; inflammation: n = 260 from 4 donors).

Oxaliplatin Increases The Responsiveness of hDRG Neurons to Cold

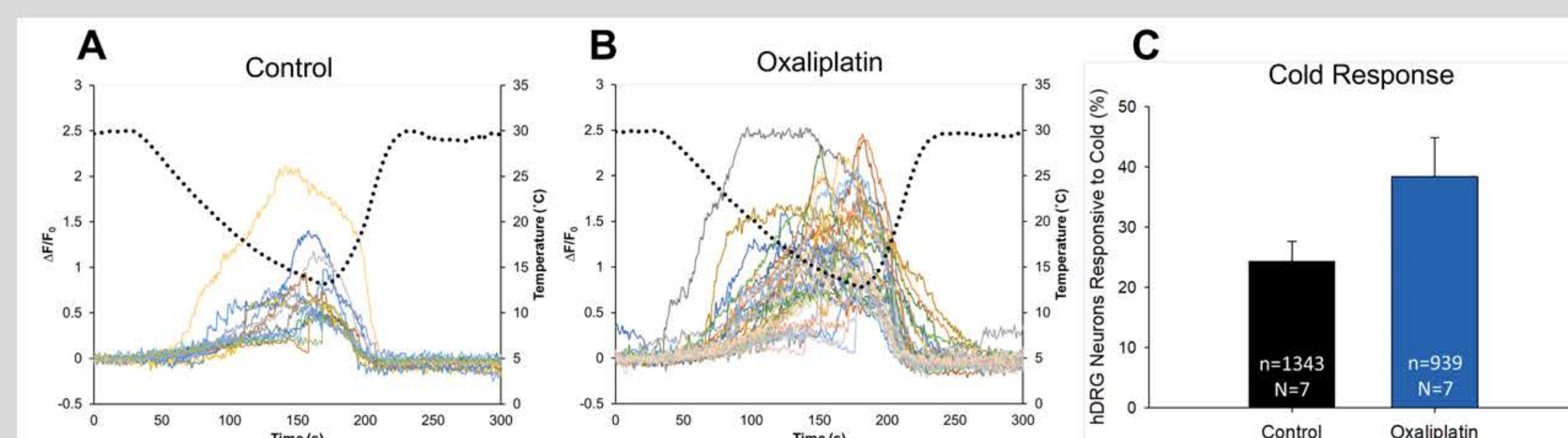


Figure 5. Chemotherapy-induced neuropathy model. **A.** Representative Ca²⁺ responses in hDRG to cold buffer application in control. **B.** Representative Ca²⁺ responses in hDRG following 24 hours of treatment with 50 µM oxaliplatin (dotted lines: temperature curve). **C.** Treatment with Oxaliplatin resulted in an increased percentage of hDRG neurons responsive to cold (control: n = 1343; Oxaliplatin: n = 939 from 7 donors).

Dose and frequency-dependence of action potential inhibition

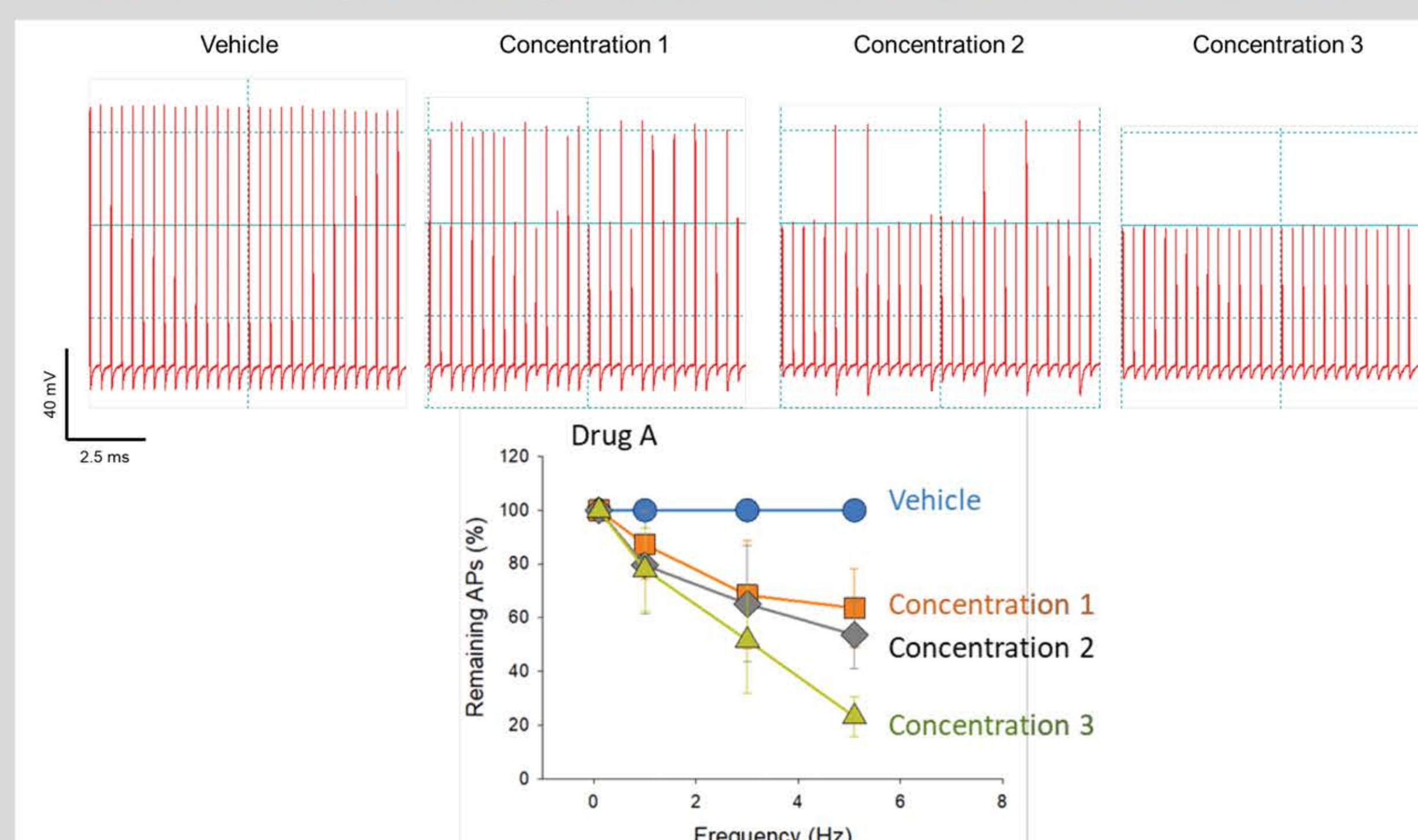


Figure 6. Dose and frequency-dependence of action potential inhibition in hDRG neurons. Individual hDRG neurons are stimulated at frequencies of 0.1, 1, 3 and 10 Hz with trains of 120 depolarization pulses delivered at 150% of firing threshold.

Distinct Nav1.7 and Nav1.8 Blocker Efficacy in Models of Pain

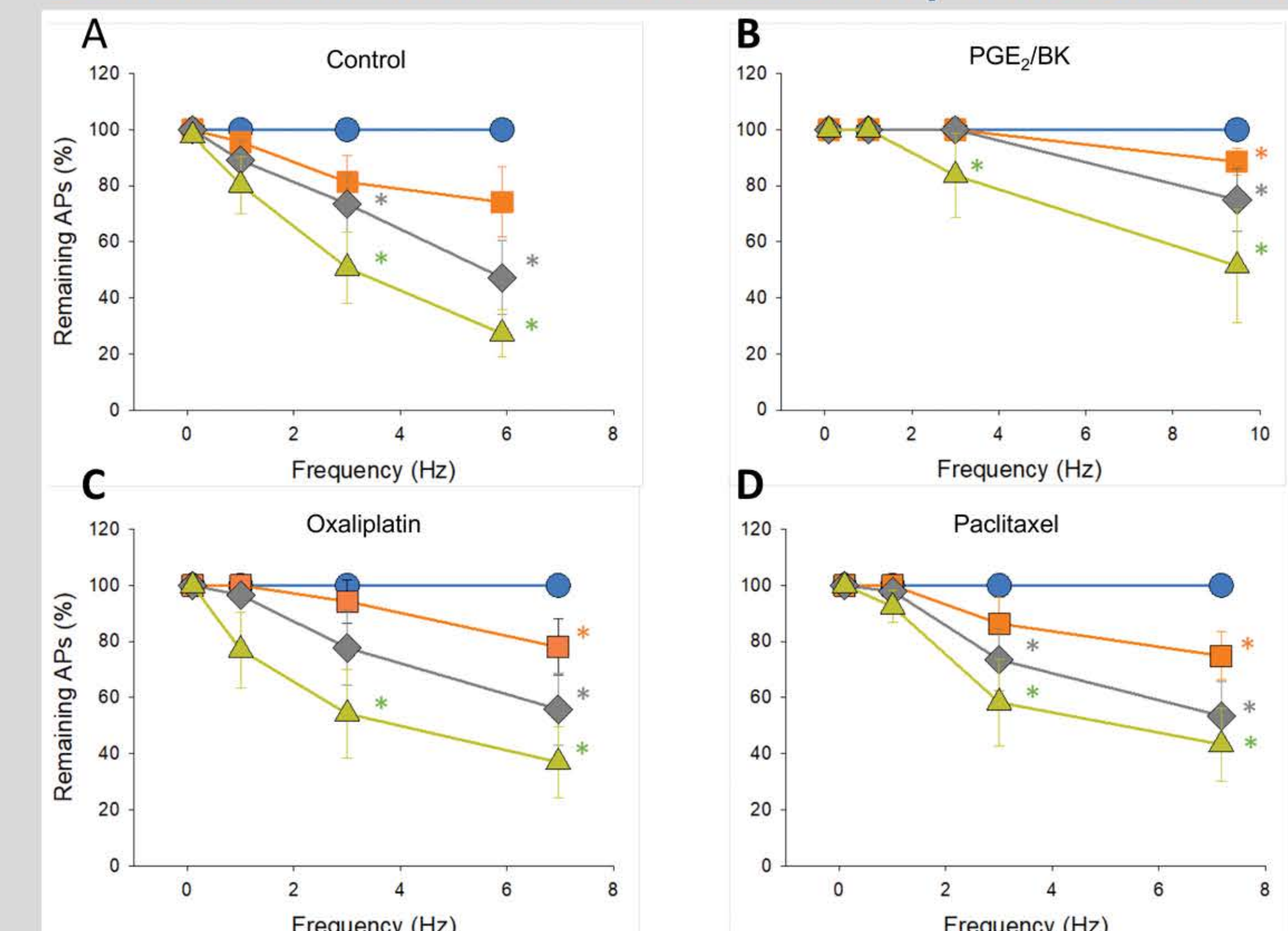


Figure 7. hDRG action potential inhibition by the Nav1.7-selective blocker PF-05089771. **A.** Control condition; n=12. **B.** 500 nM PGE₂ and 50 nM BK for 72 hours; n=9. **C.** 50 µM Oxaliplatin; n=9. **D.** 100 nM Paclitaxel; n=9 (*p<0.05).

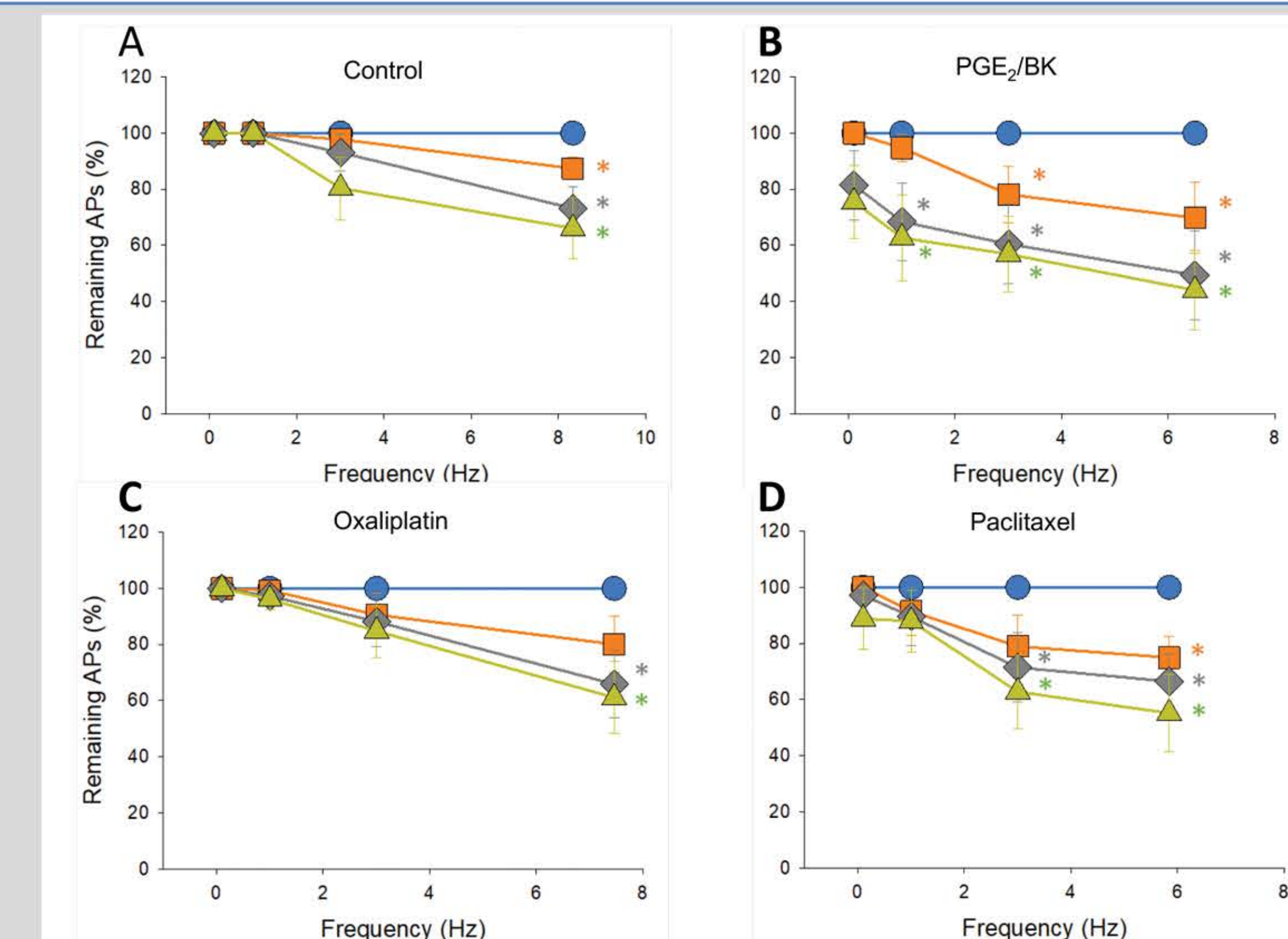


Figure 8. hDRG action potential inhibition by Nav1.8-selective blocker A-803467. **A.** Control condition; n = 9. **B.** 500 nM PGE₂ and 50 nM BK for 72 hours; n=9. **C.** 50 µM Oxaliplatin; n=9. **D.** 100 nM Paclitaxel; n=9 (*p<0.05).

Conclusions

- 1- We successfully established human *ex vivo* models for inflammation and chemotherapy-induced neuropathy.
- 2- Our Ca²⁺ imaging approach enables the high-throughput characterization of pain-induced changes in functional phenotypes of large numbers of hDRG neurons.
- 3- Our electrophysiological approach yields detailed single cell dose and frequency response relations of lead compounds.
- 4- The ability to model pain in human neurons is a powerful tool to predict the therapeutic efficacy of lead compounds under specific pain conditions.