Chemotherapy-Induced Mitochondrial Dysfunction and Neuronal Damage in iPSC-Derived Sensory Neurons

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Background

Chemotherapy-induced peripheral neuropathy (CIPN) remains a major clinical challenge, often limiting effective cancer treatment. CIPN affects a significant proportion of cancer patients, manifesting as chronic pain, numbness, and sensory deficits. Traditional research models, such as rodents, often fail to recapitulate human-specific neuronal responses to chemotherapeutics. iPSC-derived sensory neurons offer a scalable, patient-relevant system to study neurotoxicity *in vitro*. Paclitaxel and Oxaliplatin, two commonly used chemotherapeutic agents, are known to disrupt mitochondrial function and induce apoptosis in sensory neurons, which underlies CIPN. Modelling these effects in iPSC-derived sensory neurons enables mechanistic insights and supports the development of targeted interventions to alleviate CIPN.

Methods

Human-induced pluripotent stem cell (iPSC)-derived sensory neurons were exposed to different doses of Paclitaxel or Oxaliplatin to model chemotherapy-induced neurotoxicity and neuropathic pain. Cell viability was assessed by CellTiter-Glo® assay after 48 h of treatment and mitochondrial integrity via MTT assay after 96 h of treatment. Neuronal activity was monitored over time using IncuCyte® NeuroBurst, a genetically-encoded calcium indicator (GECI) in combination with the IncuCyte® Live-Cell imaging system. Expression levels of sodium channels Nav1.6 and Nav1.7 as well as pain receptor TRPV1 (Transient Receptor Potential cation channel subfamily V member 1) was quantified by RT-qPCR. Immunocytochemistry (ICC) using confocal microscopy was used to detect TRPV1, CGRP (Calcitonin Gene-Related Peptide), Tubulin and MAP2 (Microtubule-Associated Protein 2).

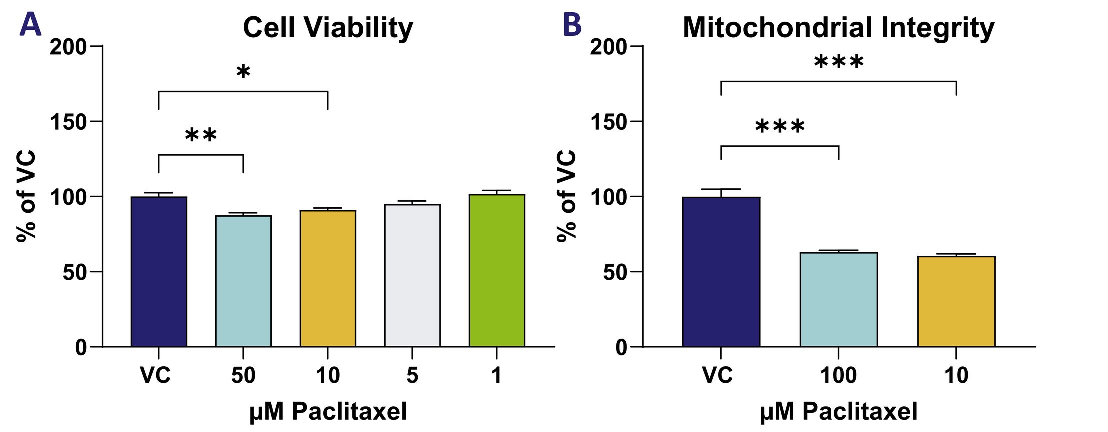
Results

We employed iPSC-derived sensory neurons to model CIPN and evaluated neurotoxicity induced by Paclitaxel and Oxaliplatin. Using CellTiter-Glo® and MTT assays, we observed a reduction in neuronal viability and mitochondrial integrity, indicating mitochondrial dysfunction and cytotoxicity. Neuronal activity was affected by both compounds. Sodium channel and TRPV1 expression remained unchanged with Paclitaxel treatment but was reduced following Oxaliplatin exposure.

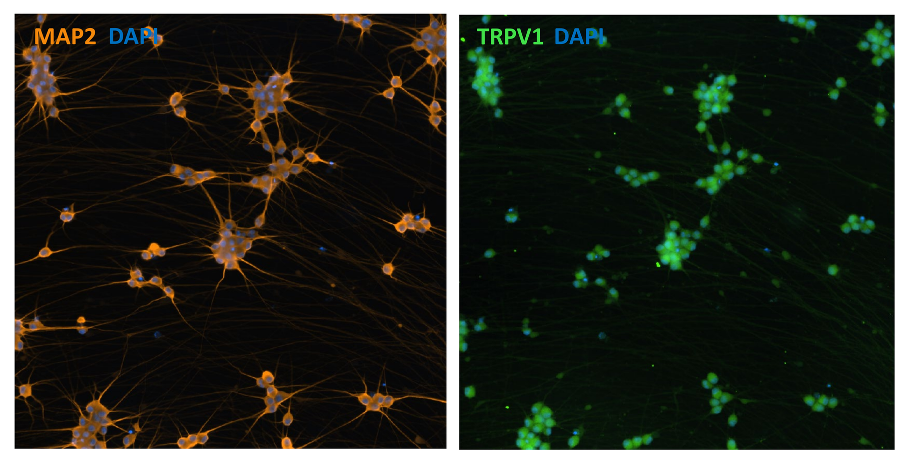
Conclusion

The current study validates iPSC-derived sensory neurons as a translationally relevant platform for mechanistic studies and preclinical screening of neuroprotective strategies against CIPN.

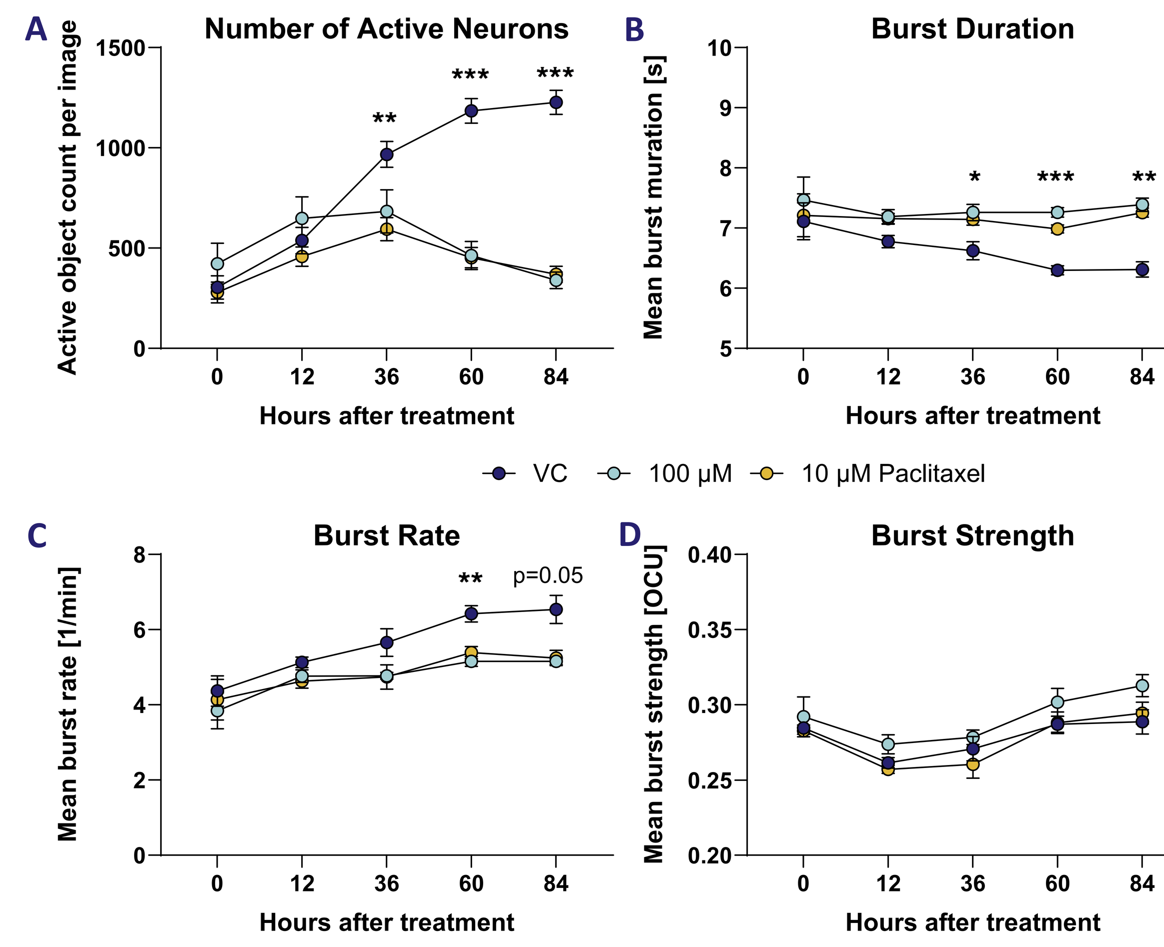
Paclitaxel



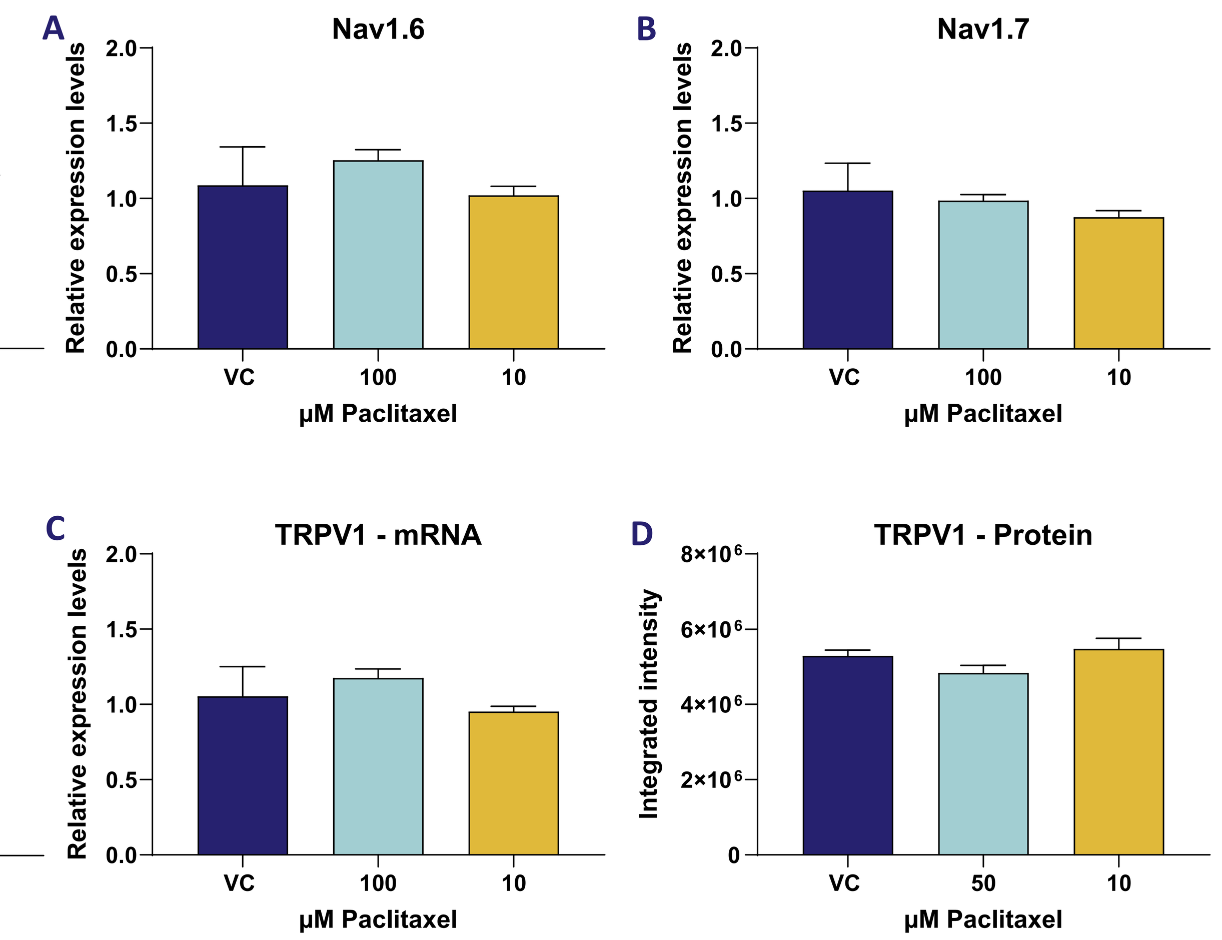
▲**Figure 1: Reduced cell viability and mitochondrial integrity in sensory neurons treated with Paclitaxel.** Cell viability was assessed after a 48-h treatment of sensory neurons (DIV15) by CellTiter-Glo® assay (**A**) and mitochondrial integrity (**B**) after 96 h of treatment (DIV21) by MTT assay. Data are presented as percent of vehicle control (% VC); Mean + SEM; n = 4-5 per group. One-way ANOVA with Dunnett's *post hoc* test. *p <0.05; **p <0.01; ***p <0.001.



Representative images of MAP2 and TRPV1 staining

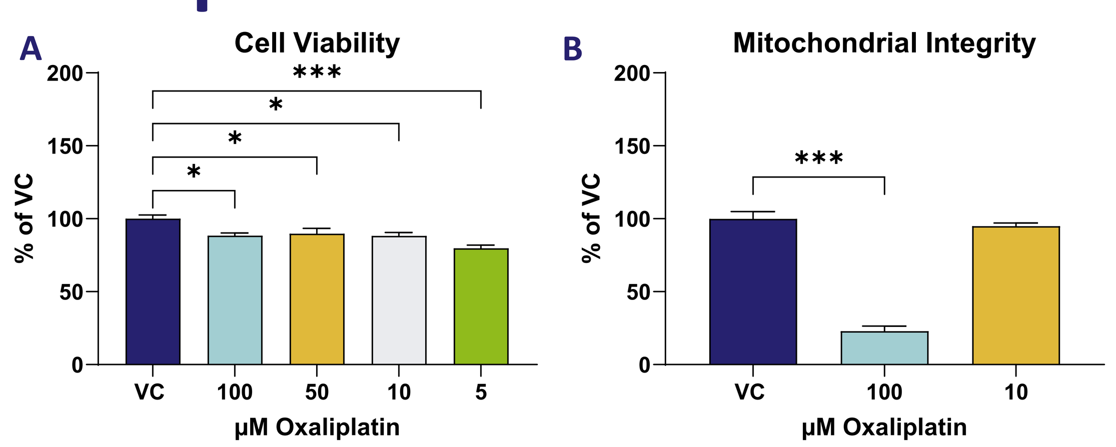


▲**Figure 2: Altered neuronal activity in sensory neurons treated with Paclitaxel.** Neuronal activity was assessed over time using IncuCyte® NeuroBurst after treatment of sensory neurons on DIV17 by IncuCyte® Live-Cell imaging analysis system. Number of active neurons per well (**A**), mean burst duration (**B**), mean burst rate (**C**) and mean burst strength (**D**). Mean + SEM; n = 4 per group. Two-way ANOVA with Dunnett's *post hoc* test. *p <0.05; **p <0.01; ***p <0.001.

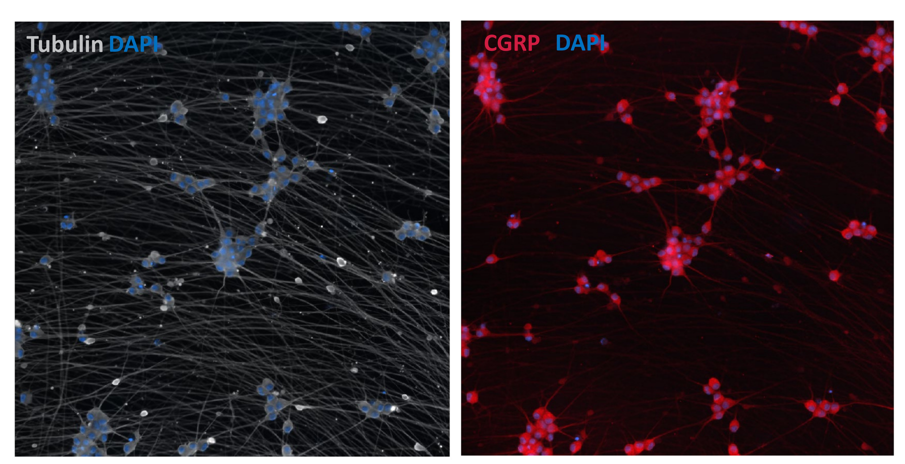


▲**Figure 3: Unaffected expression levels of sodium channels and TRPV1 in sensory neurons treated with Paclitaxel.** Relative expression levels of Nav1.6 (**A**), Nav1.7 (**B**) and TRPV1 (**C, D**) was assessed after a 48-h treatment of sensory neurons (DIV15) by RT-qPCR or ICC. Data are presented as relative expression levels; VC = vehicle control; Mean + SEM; n = 4 per group. One-way ANOVA with Dunnett's *post hoc* test.

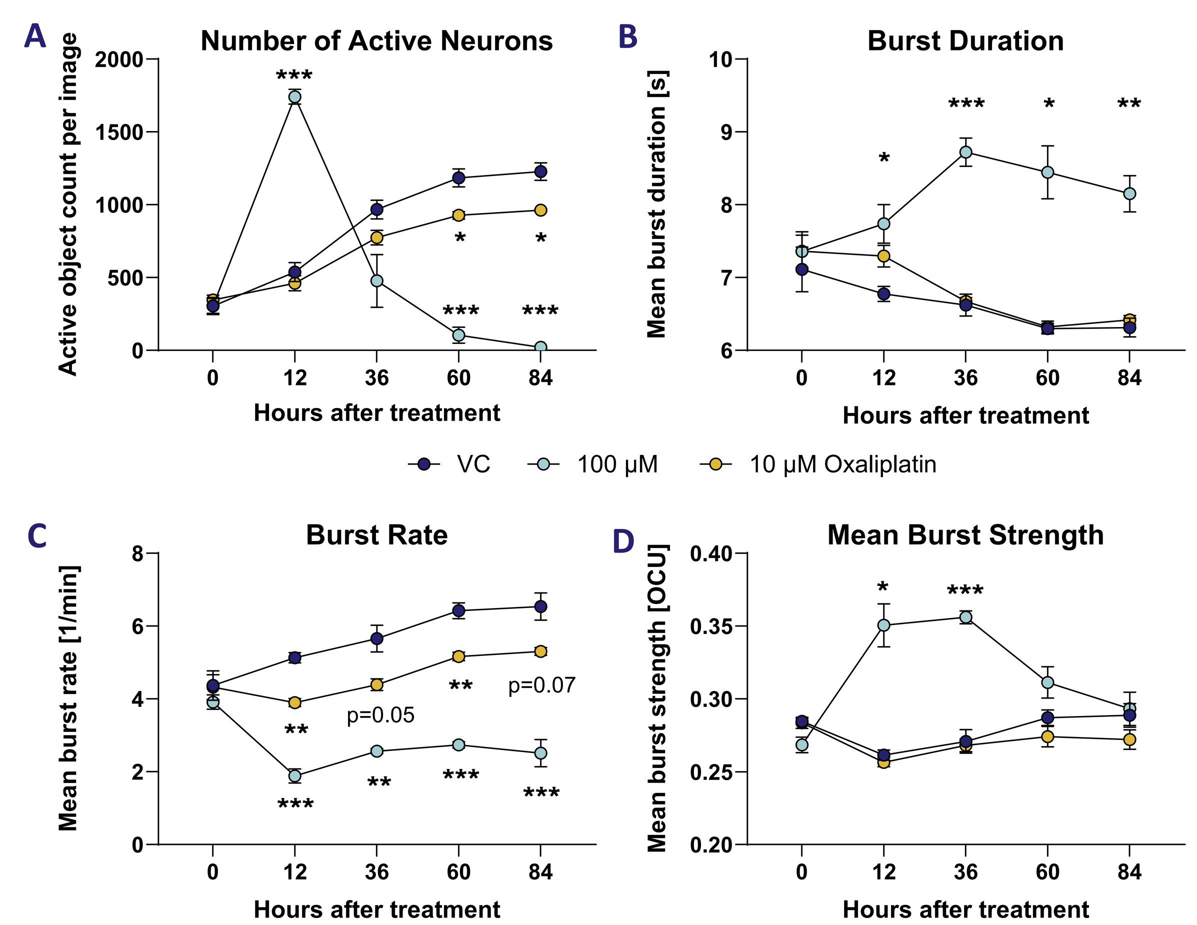
Oxaliplatin



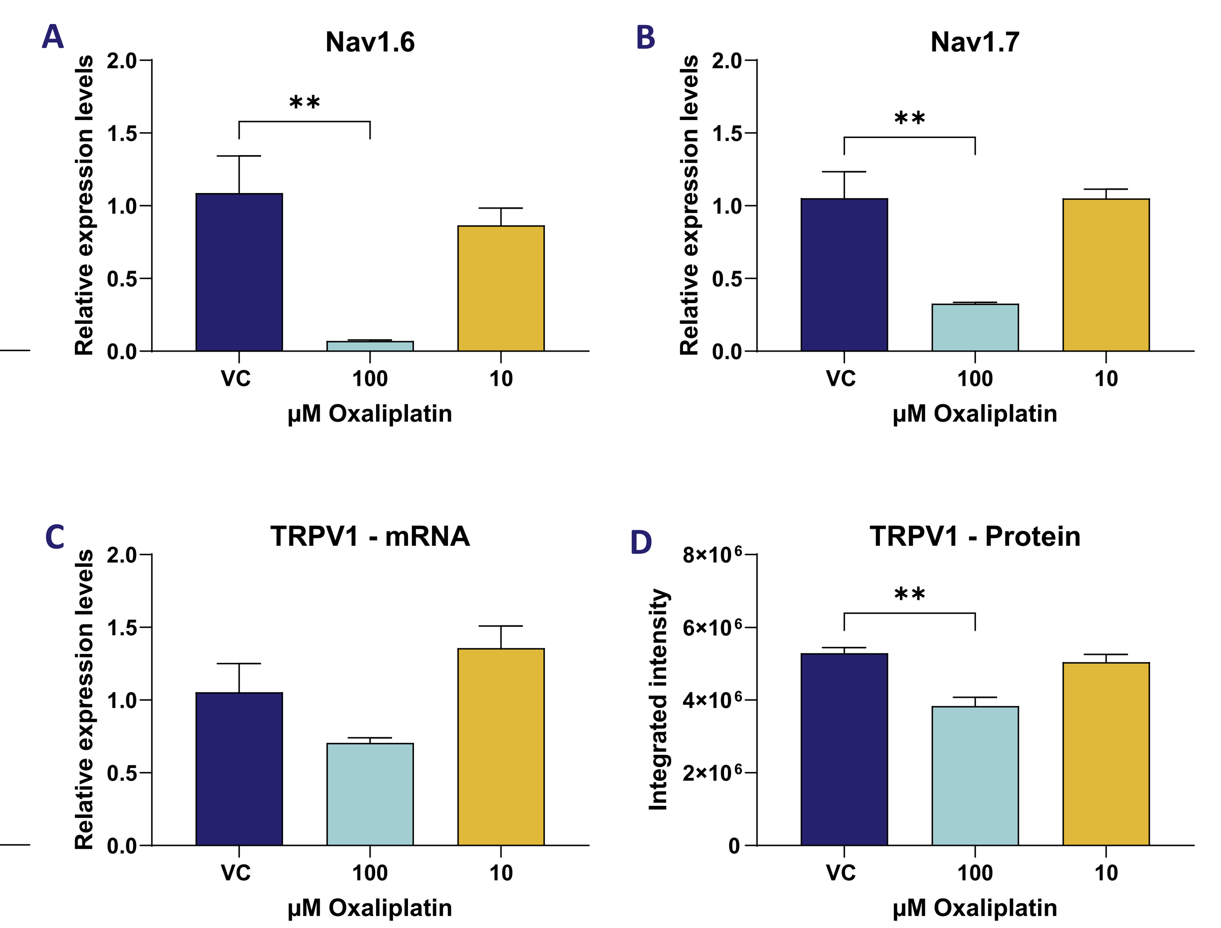
▲**Figure 4: Reduced cell viability and mitochondrial integrity in sensory neurons treated with Oxaliplatin.** Cell viability was assessed after a 48-h treatment of sensory neurons (DIV15) by CellTiter-Glo® assay (**A**) and mitochondrial integrity (**B**) after 96 h of treatment (DIV21) by MTT assay. Data are presented as percent of vehicle control (% VC); Mean + SEM; n = 4-5 per group. One-way ANOVA with Dunnett's *post hoc* test. *p <0.05; ***p <0.001.



Representative images of Tubulin and CGRP staining



▲**Figure 5: Altered neuronal activity in sensory neurons treated with Oxaliplatin.** Neuronal activity was assessed over time using IncuCyte® NeuroBurst after treatment of sensory neurons on DIV17 by IncuCyte® Live-Cell imaging analysis system. Number of active neurons per well (**A**), mean burst duration (**B**), mean burst rate (**C**) and mean burst strength (**D**). Mean + SEM; n = 4 per group. Two-way ANOVA with Dunnett's *post hoc* test. *p <0.05; **p <0.01; ***p <0.001.



▲**Figure 6: Decreased expression levels of sodium channels and TRPV1 in sensory neurons treated with Oxaliplatin.** Relative expression levels of Nav1.6 (**A**), Nav1.7 (**B**) and TRPV1 (**C, D**) was assessed after a 48-h treatment of sensory neurons (DIV15) by RT-qPCR or ICC. Data are presented as relative expression levels; VC = vehicle control; Mean + SEM; n = 4 per group. One-way ANOVA with Dunnett's *post hoc* test. **p <0.01.

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