

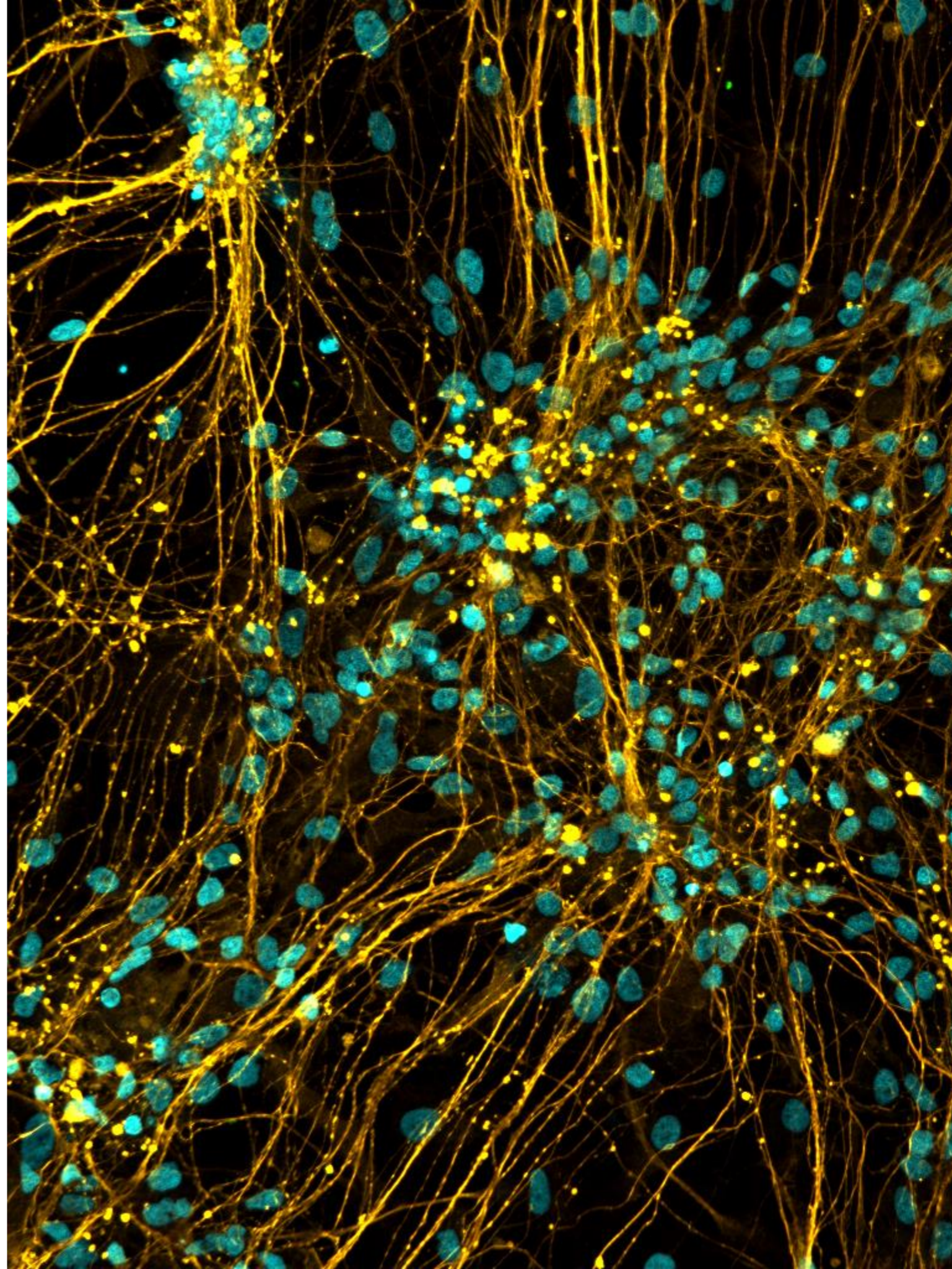
Potential applications of hiPSC-derived Peripheral Sensory Neurons in vitro models

Marília Zaluar Guimarães

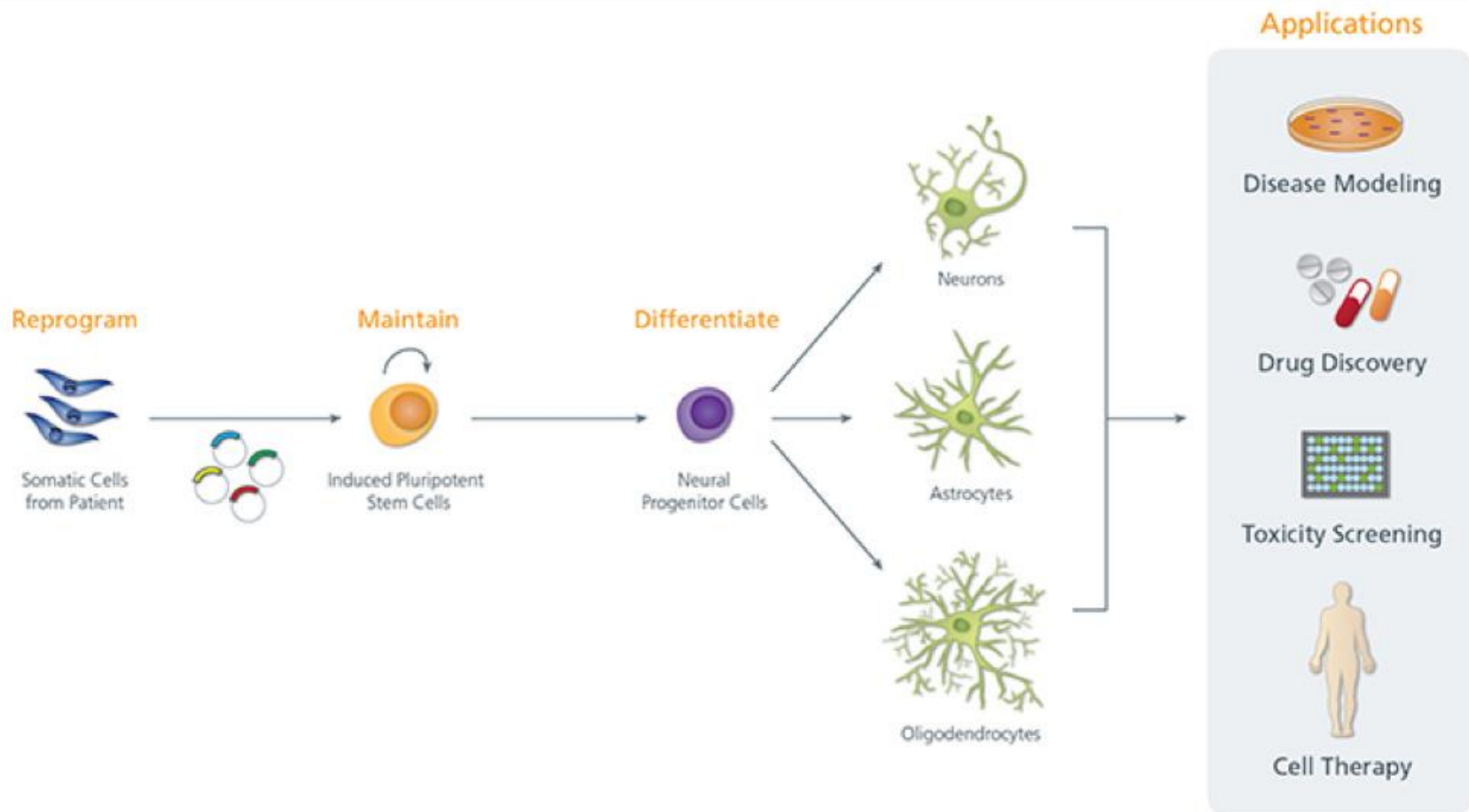
Instituto D'Or de Pesquisa e Ensino
Universidade Federal do Rio de Janeiro

Why peripheral sensory neurons?

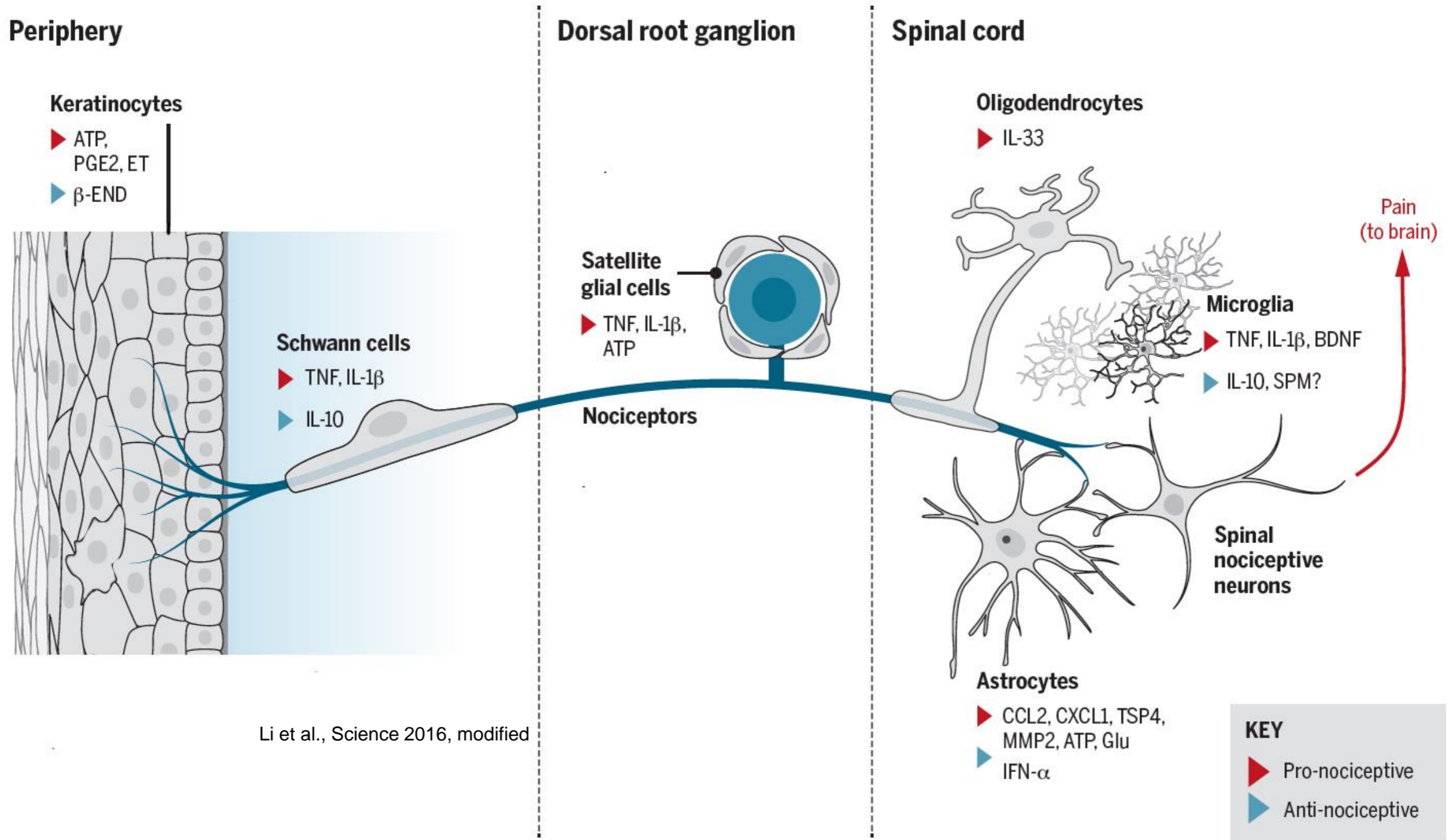
- To study its development and function
- To develop alternative assays to screen for irritants and analgesics acting locally
- To study human pathologies in which they are involved, such as neuropathic pain



Human induced pluripotent stem cells (hiPSCs)

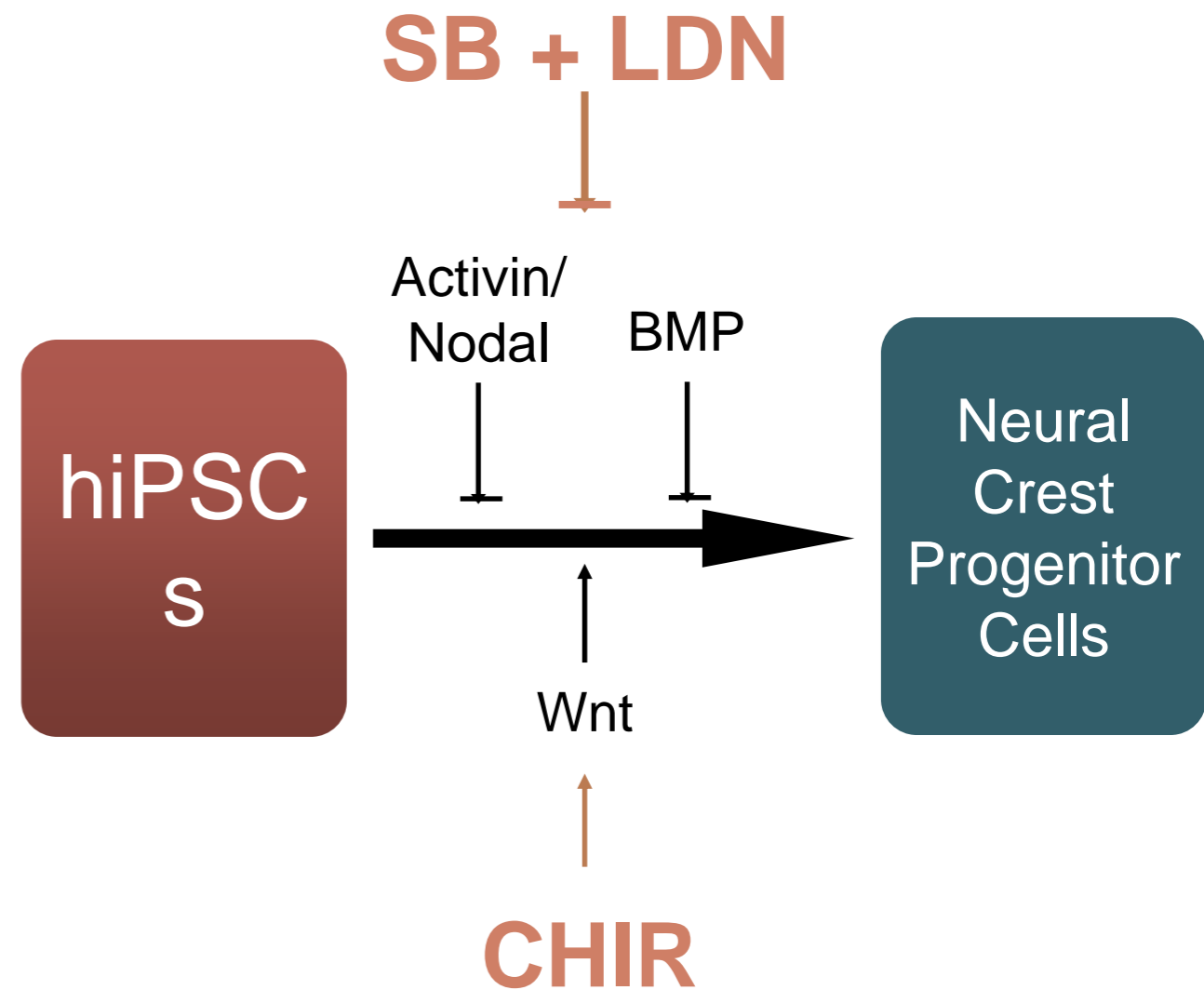
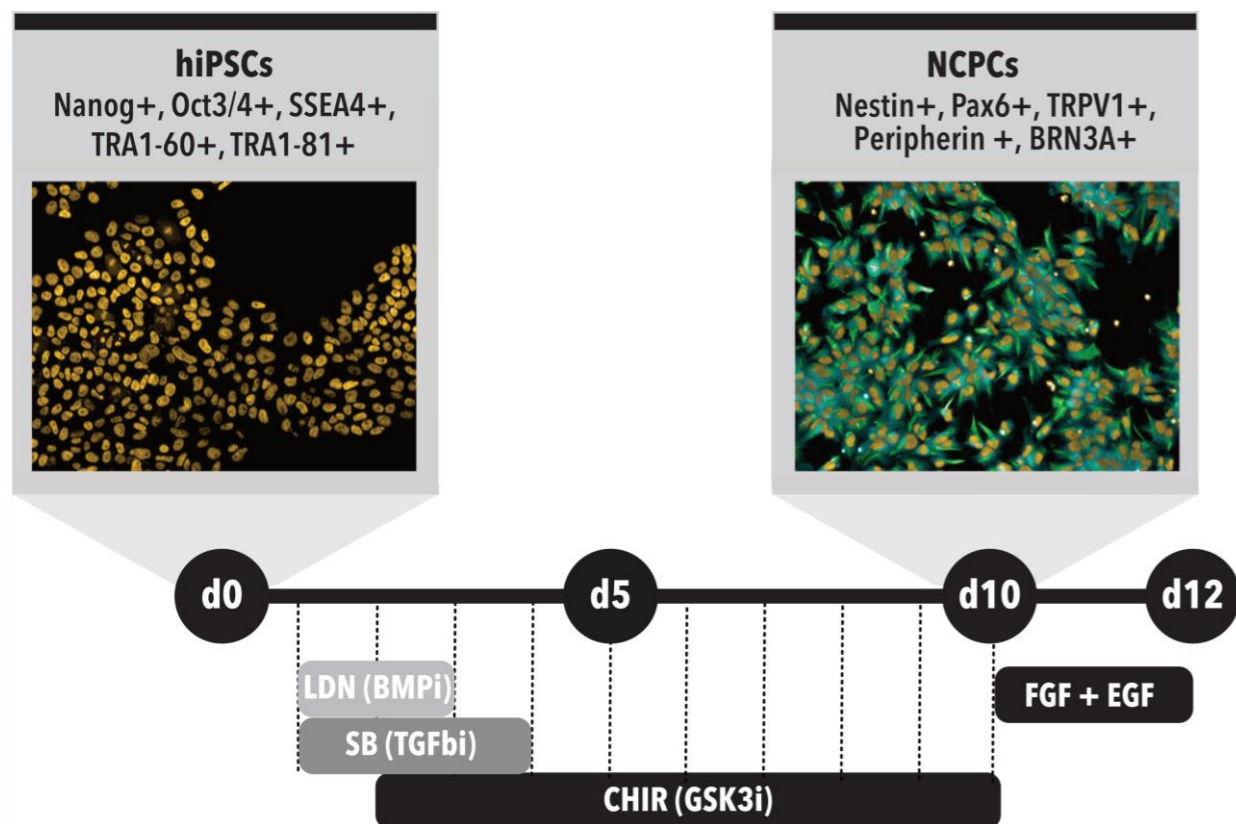


Nociceptors and keratinocytes

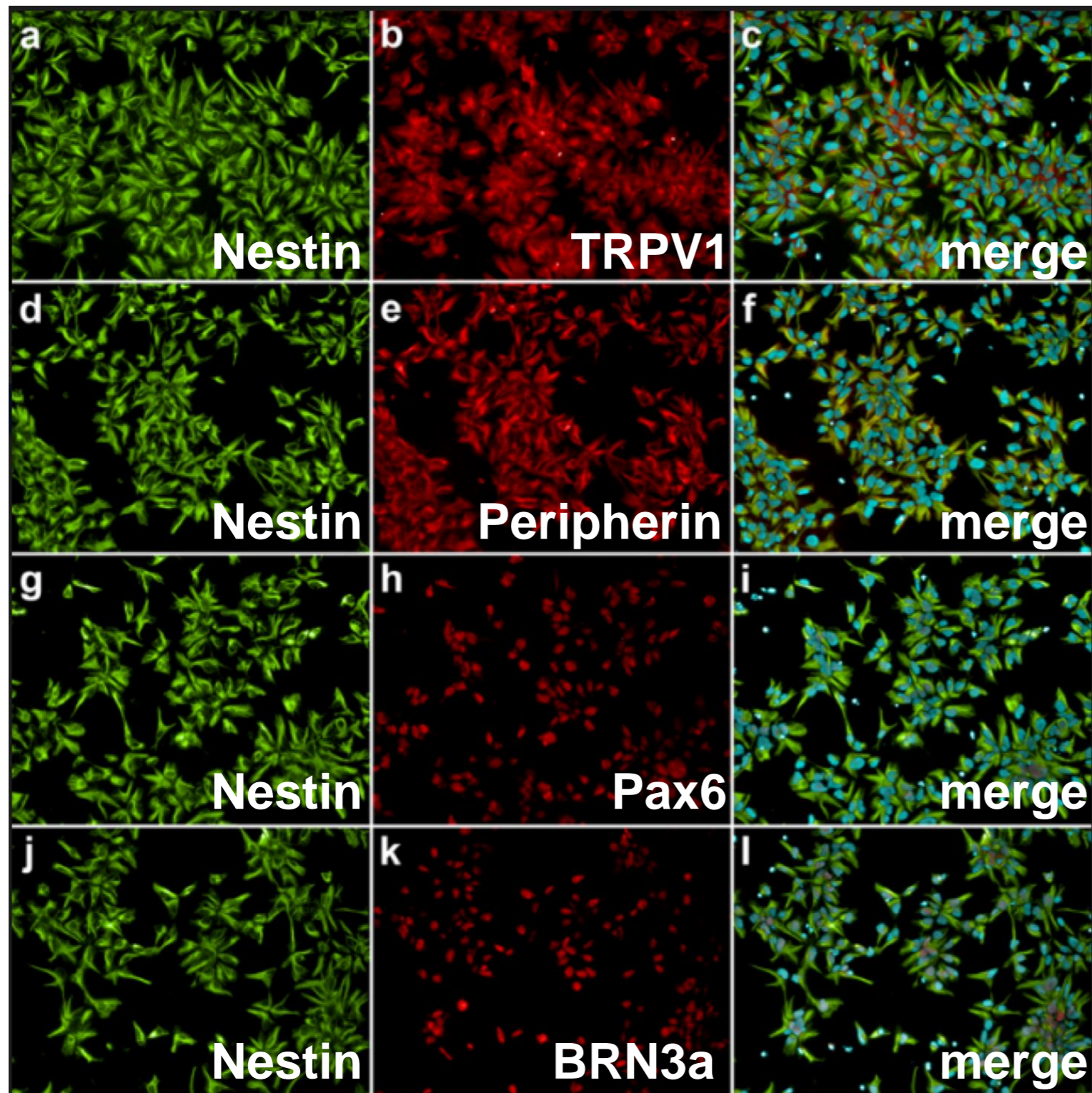


Li et al., Science 2016, modified

Protocol scheme: From hiPSCs to neural crest progenitor cells (NCPCs)



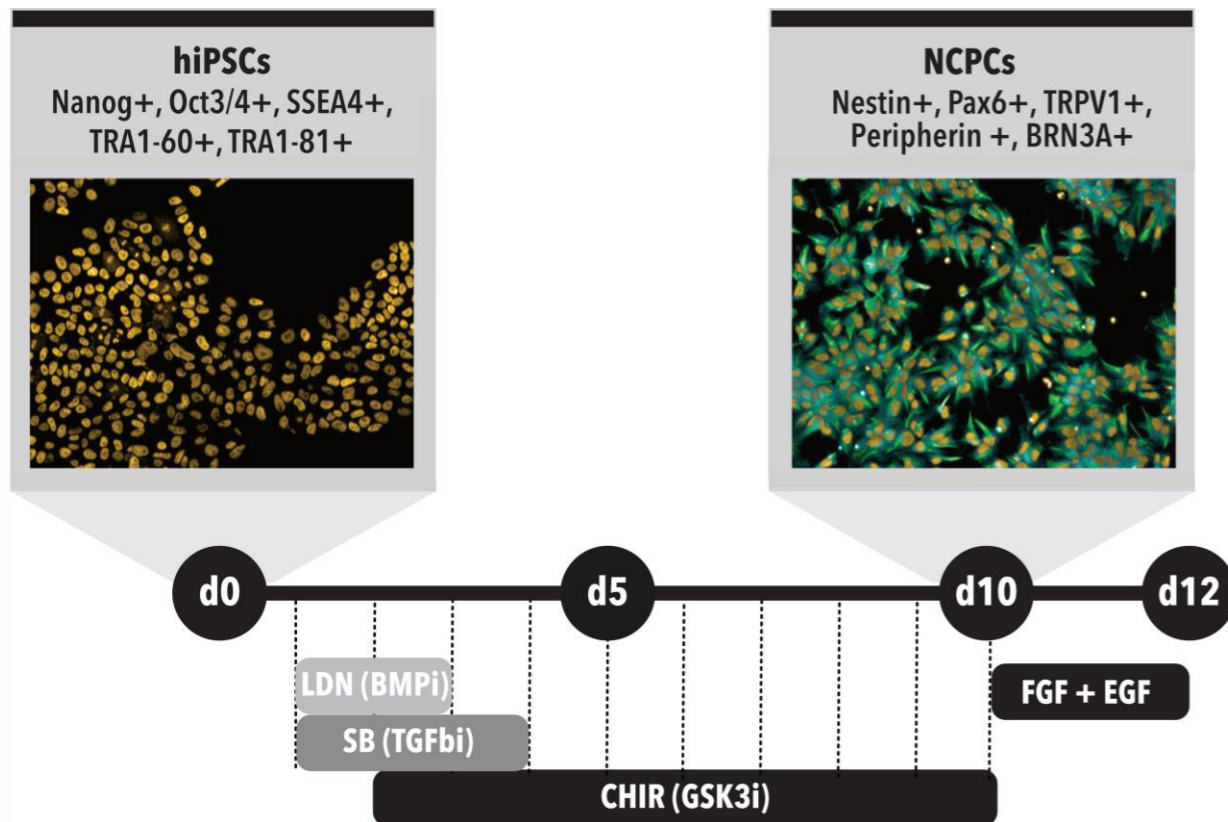
Neural Crest Progenitor Cells (NCPCs) markers



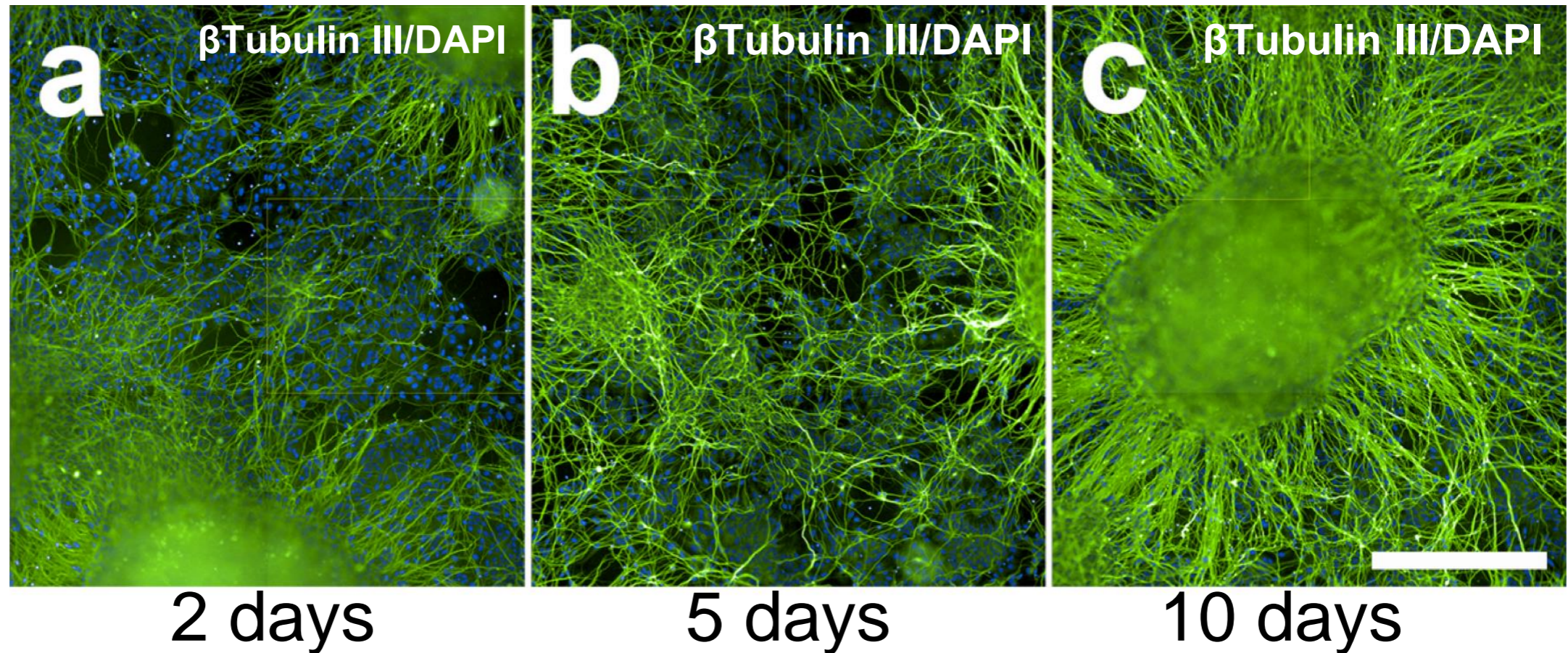
Key:

- Nestin: intermediate filament; neural stem cells
- TRPV1: membrane receptor; nociceptor
- Peripherin: intermediate filament; peripheral neurons
- Pax6: transcription factor; regulates neurogenesis
- BRN3a: transcription factor; present in peripheral sensory neurons

Protocol scheme

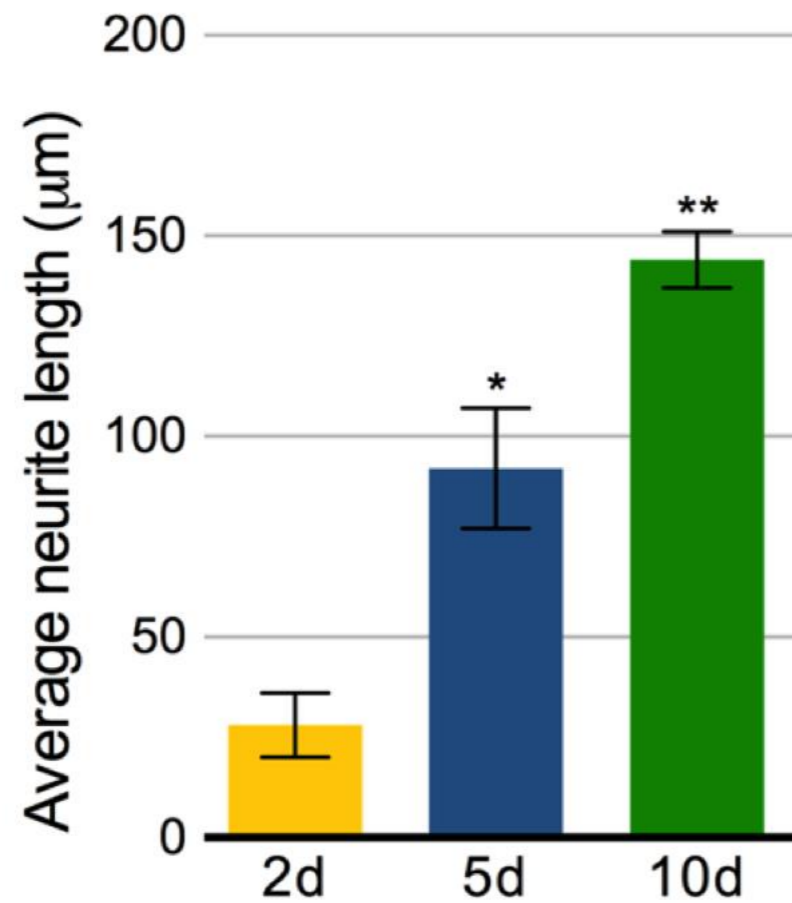


Peripheral sensory neurons (PSN) and human epidermic keratinocytes (neonatal - HEKn)-conditioned medium

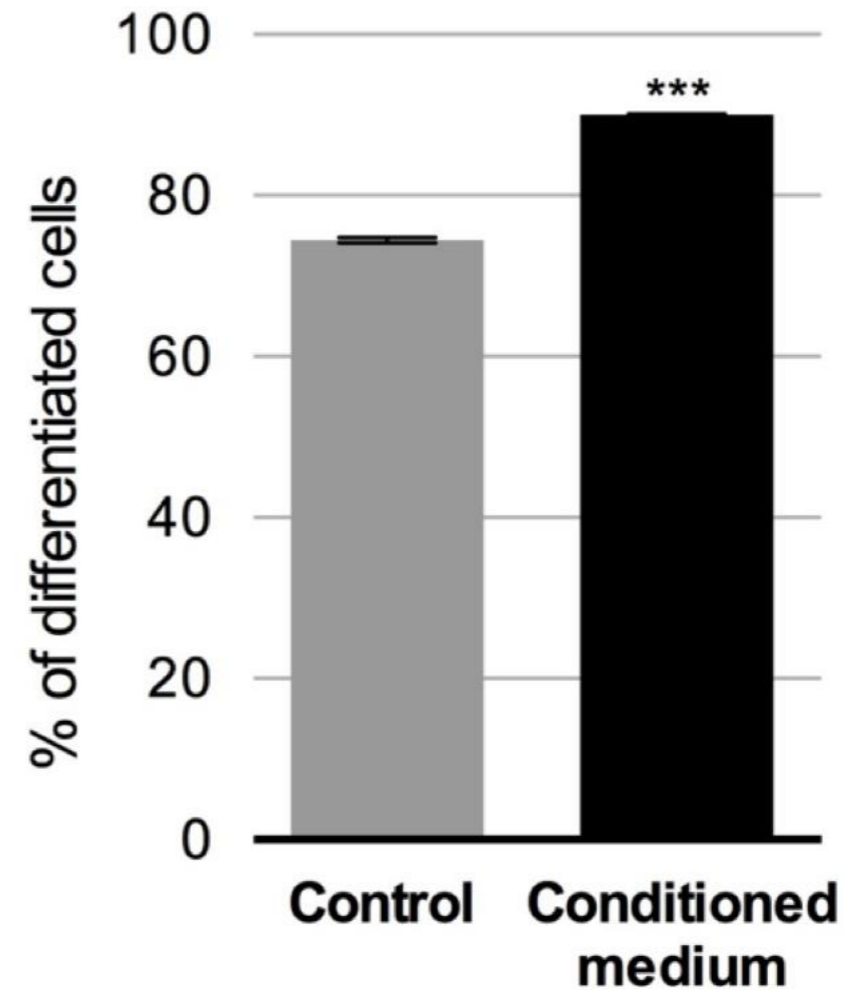


PSN and HEKn-conditioned medium: Growth and Differentiation

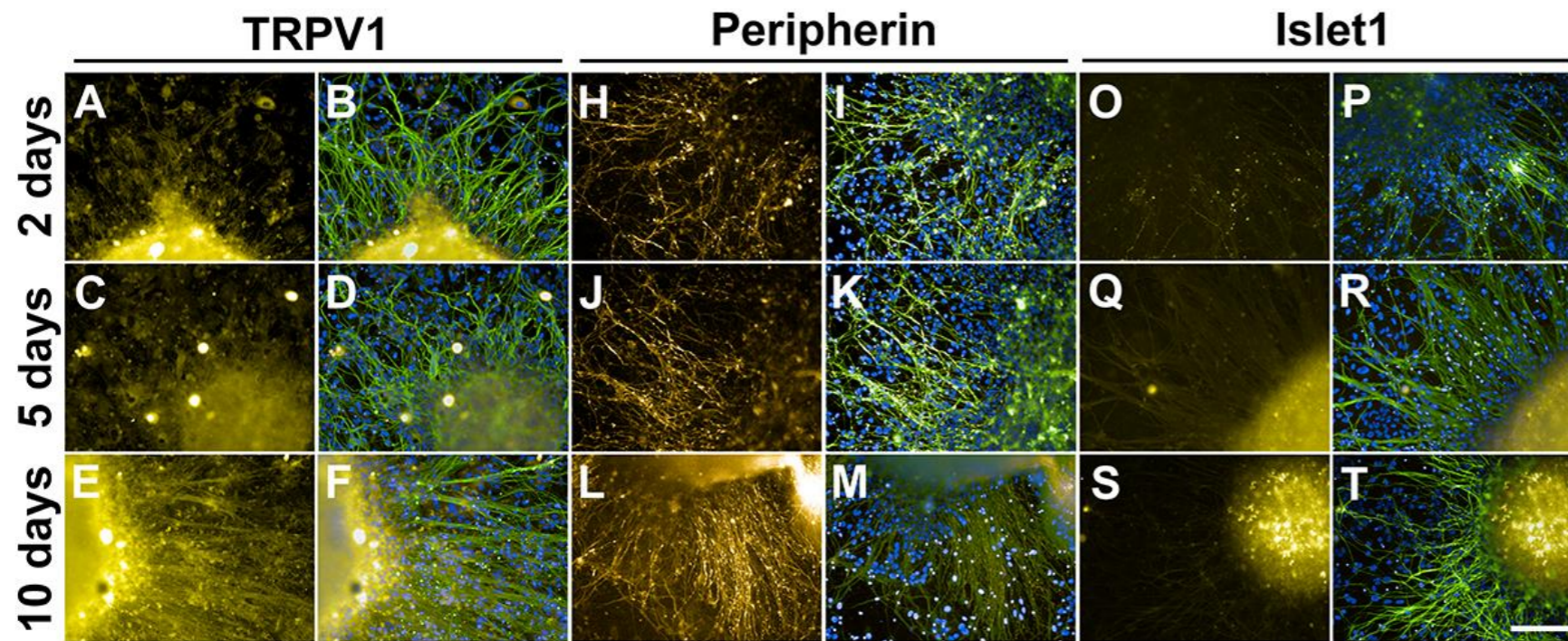
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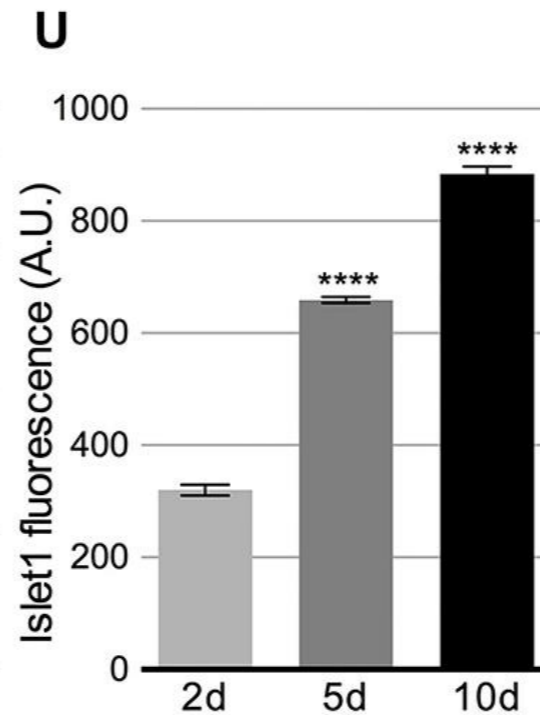
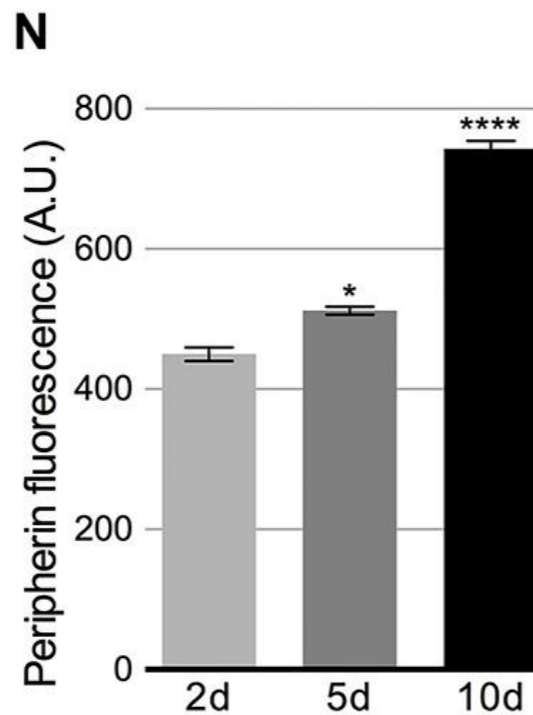
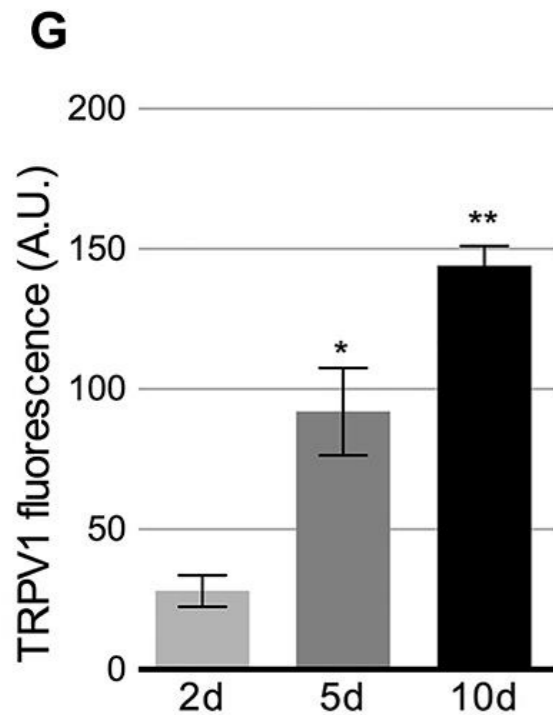
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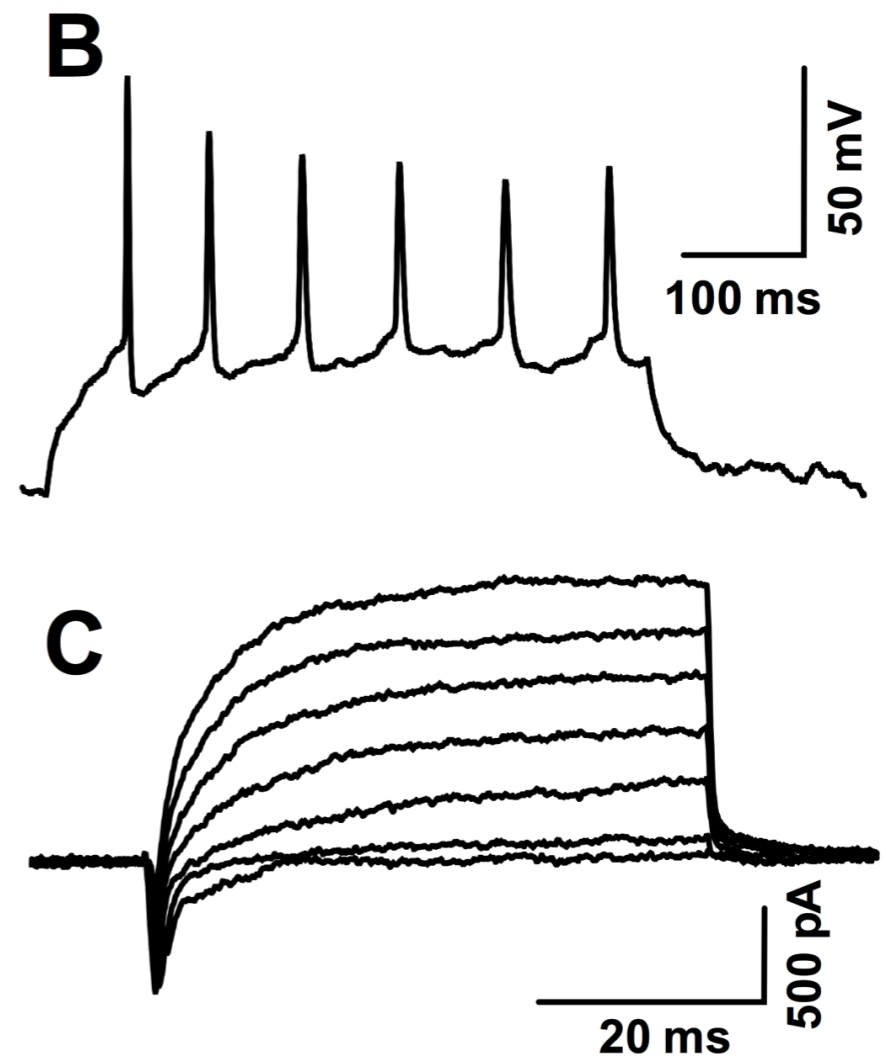
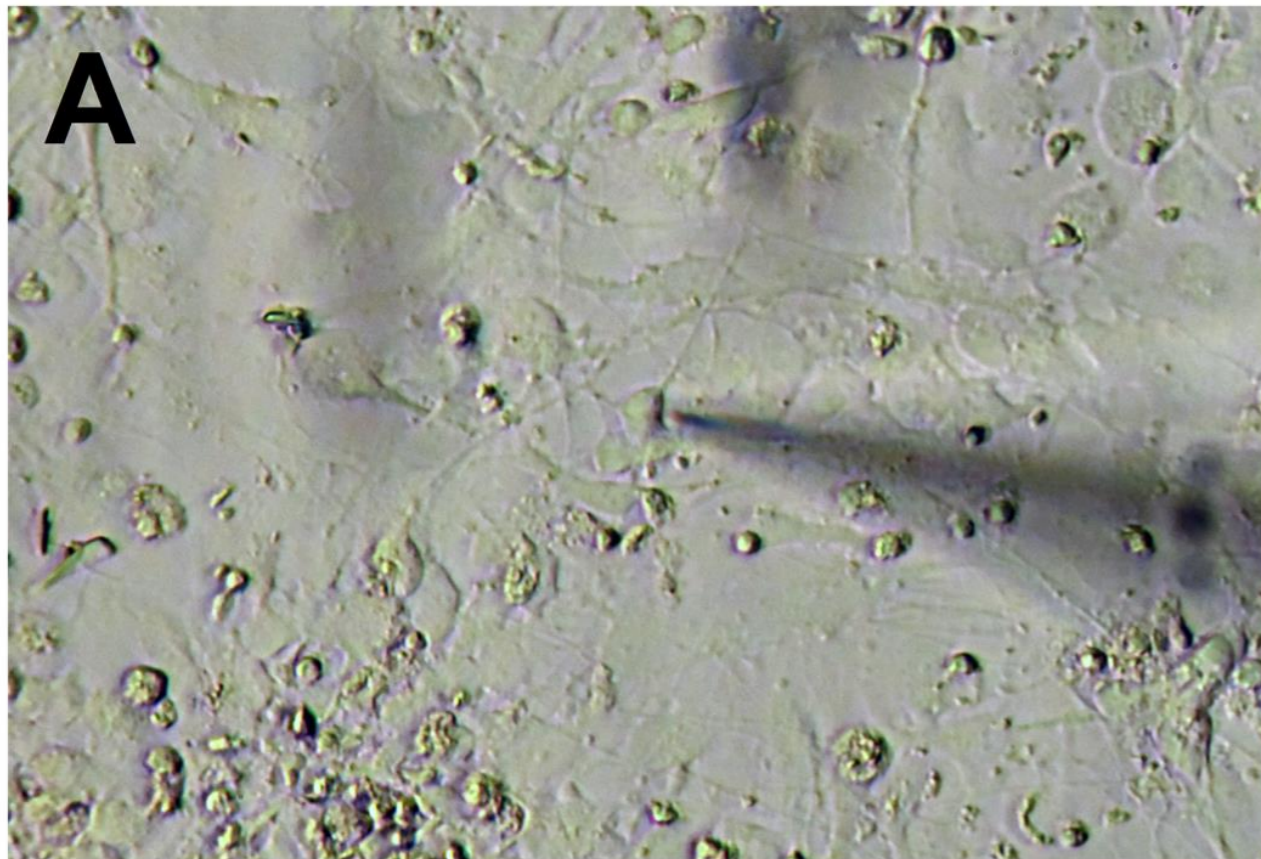
Neurons - characterization



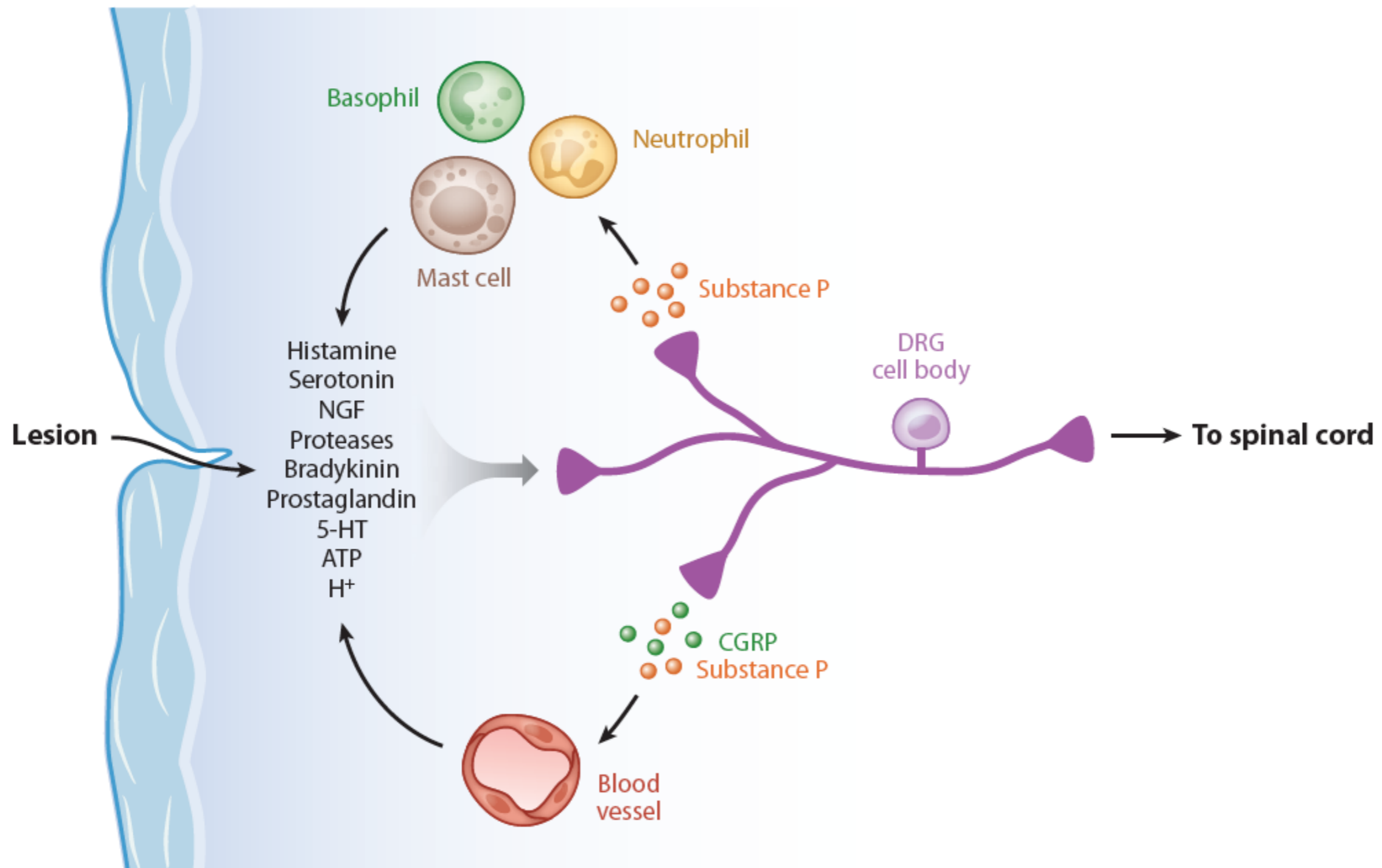
Key:
 TRPV1: membrane receptor; nociceptor
 Peripherin: intermediate filament; peripheral neurons
 Islet1: transcription factor; present in peripheral sensory neurons



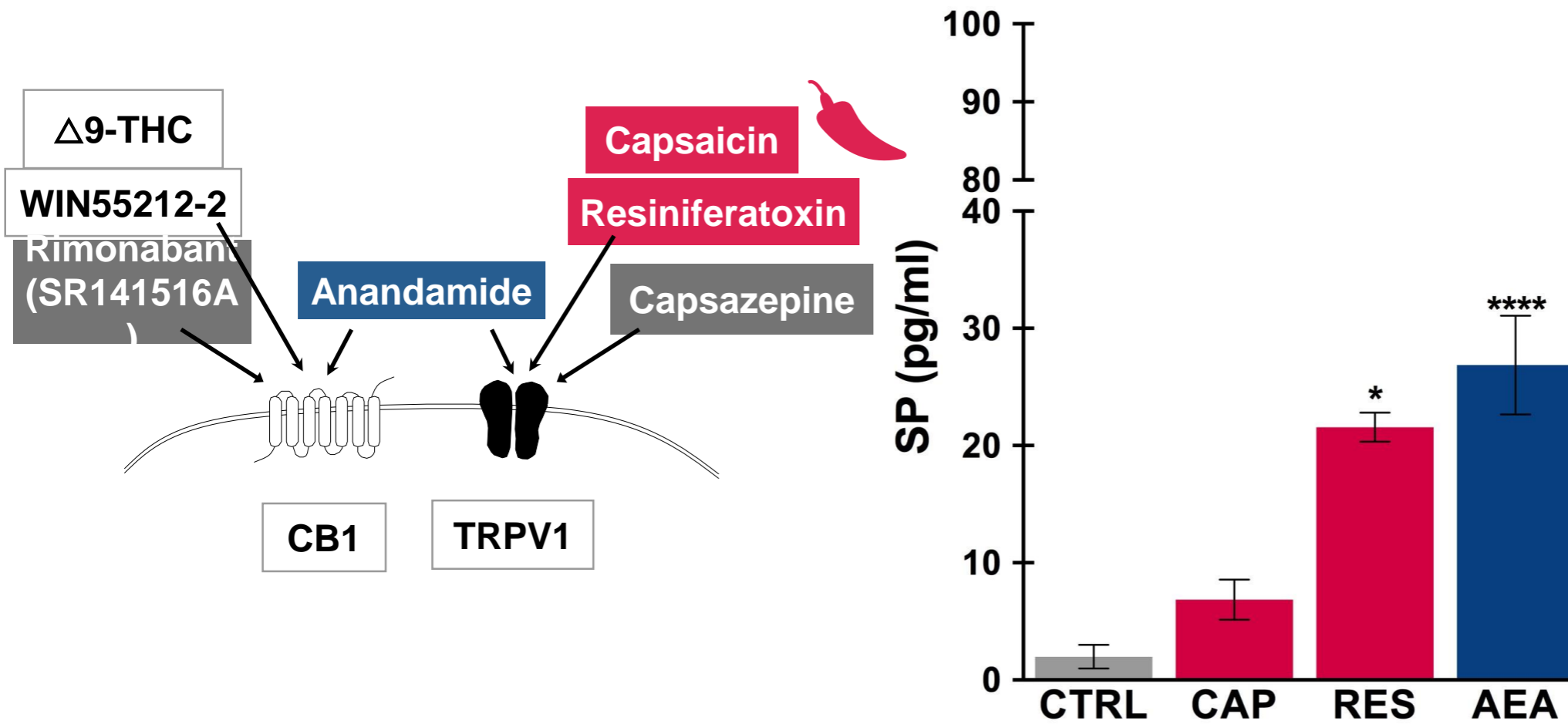
Neurons - characterization (whole-cell patchcamp)



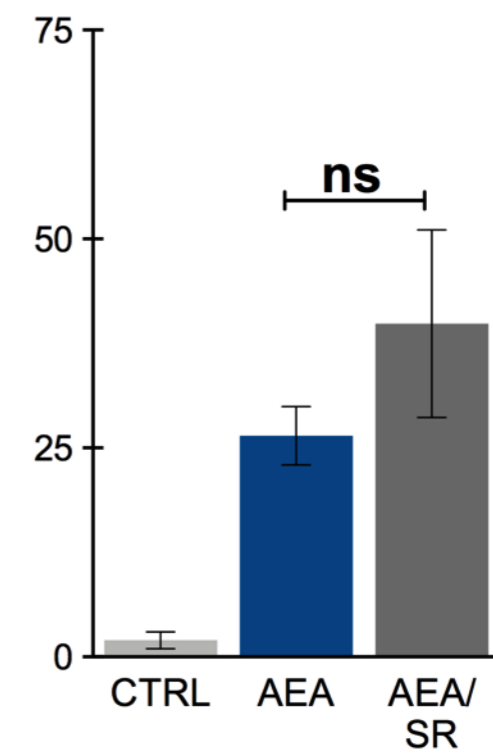
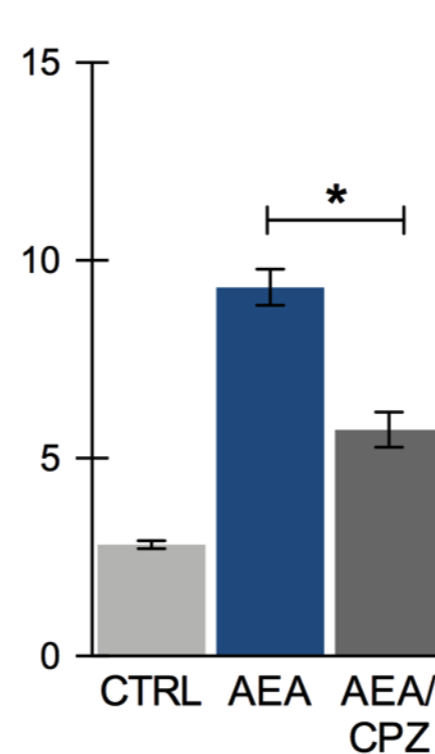
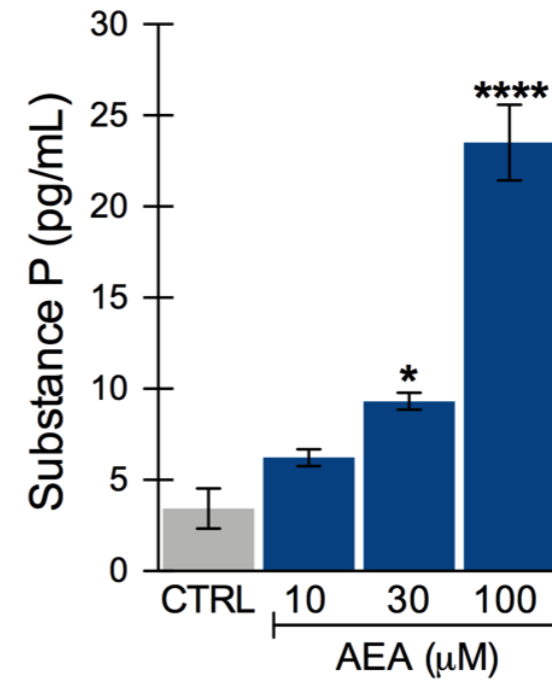
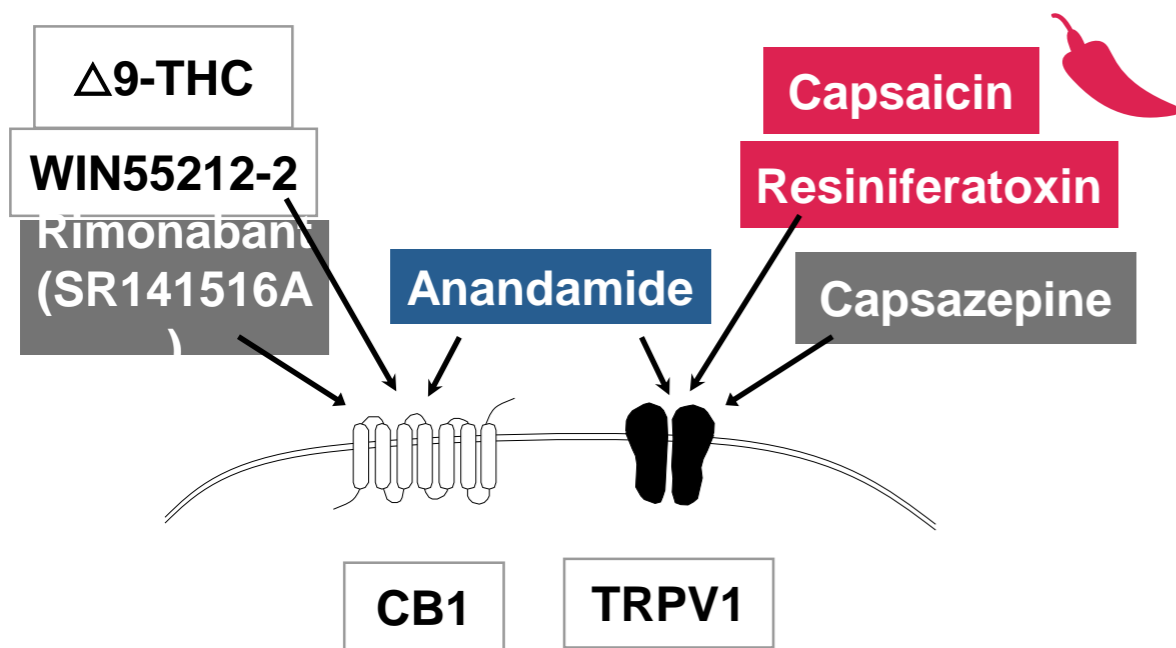
PSN activation assay: Substance P



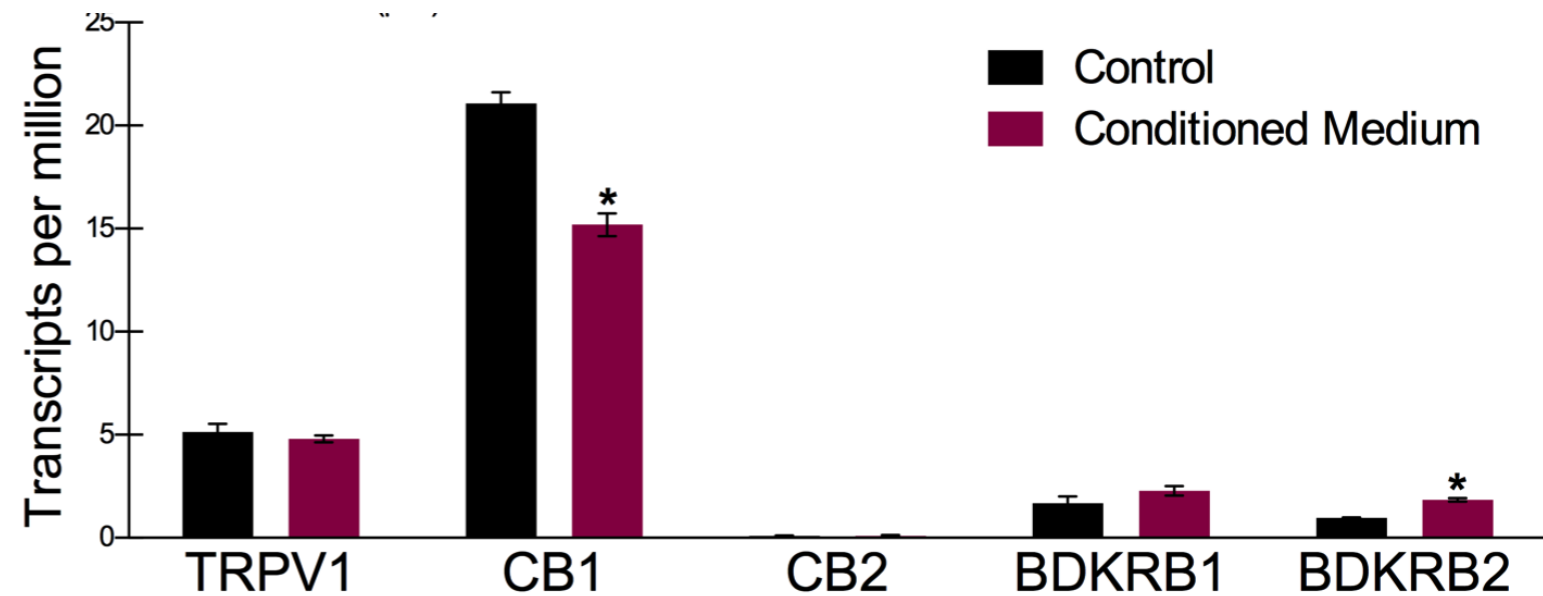
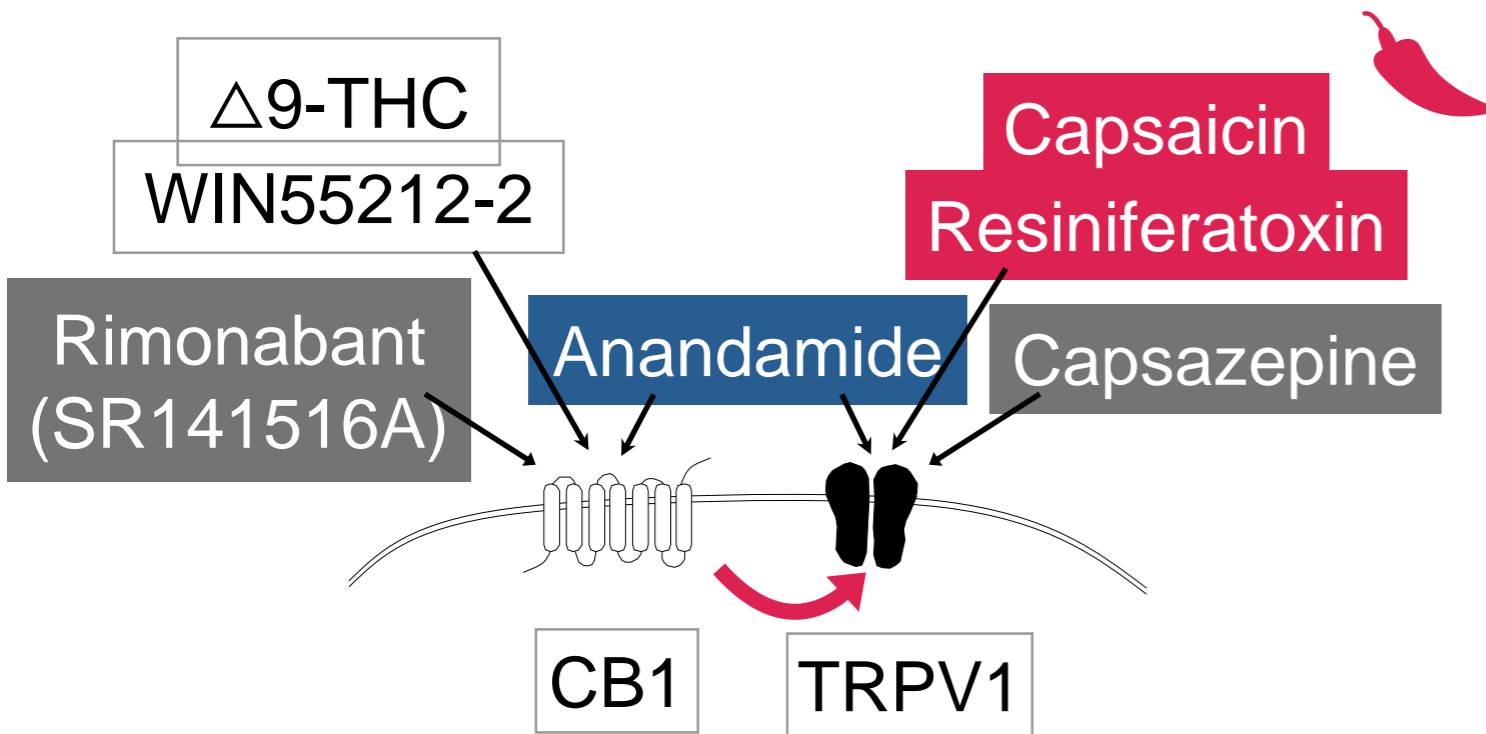
PSN functional assays: Substance P release



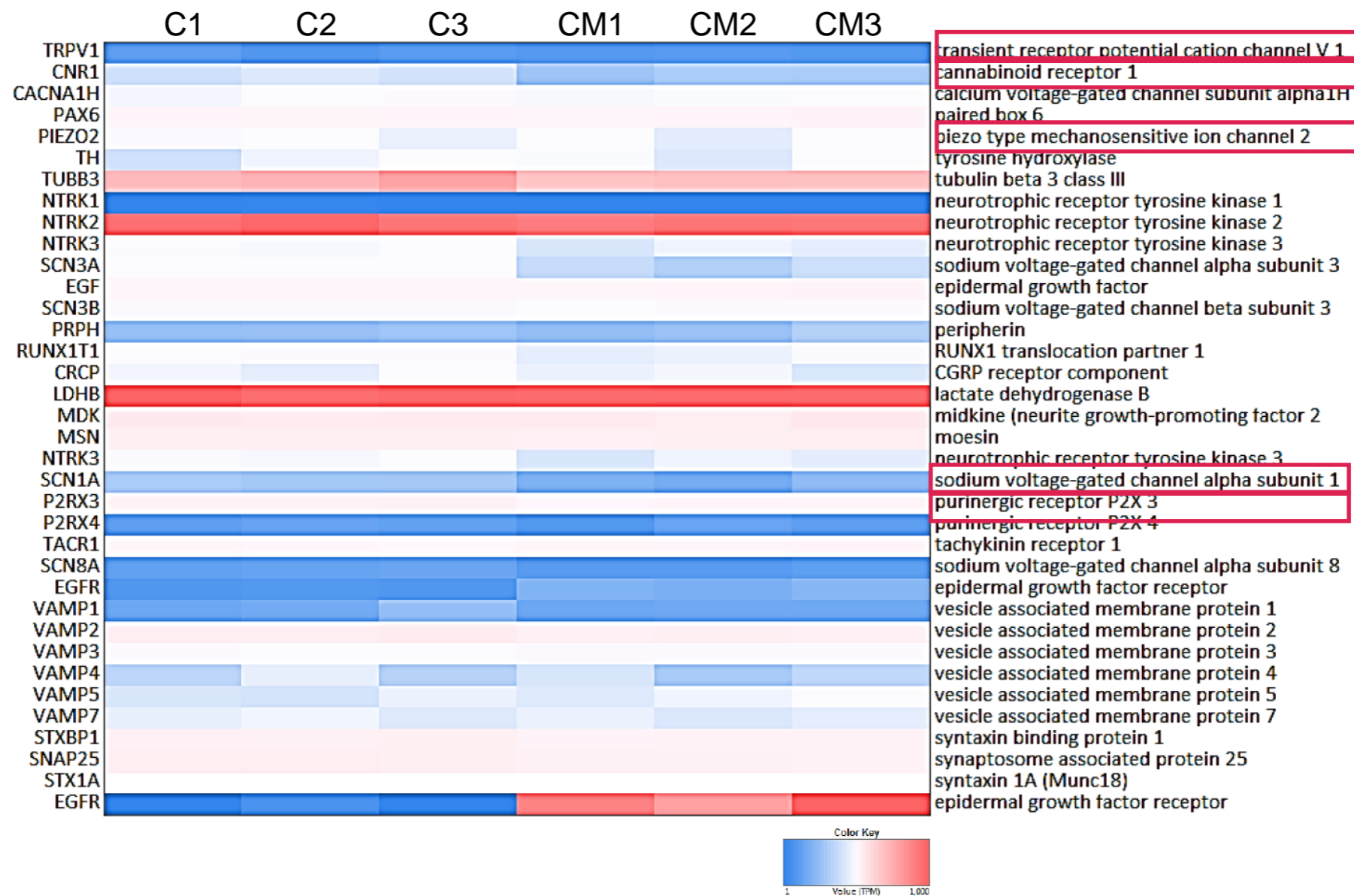
PSN functional assays: Substance P release - Anandamide (AEA)



Vanilloid-Cannabinoid pharmacology: Transcriptomics



RNA-Seq transcriptomic analysis





Generation of iPSC-Derived Human Peripheral Sensory Neurons Releasing Substance P Elicited by TRPV1 Agonists

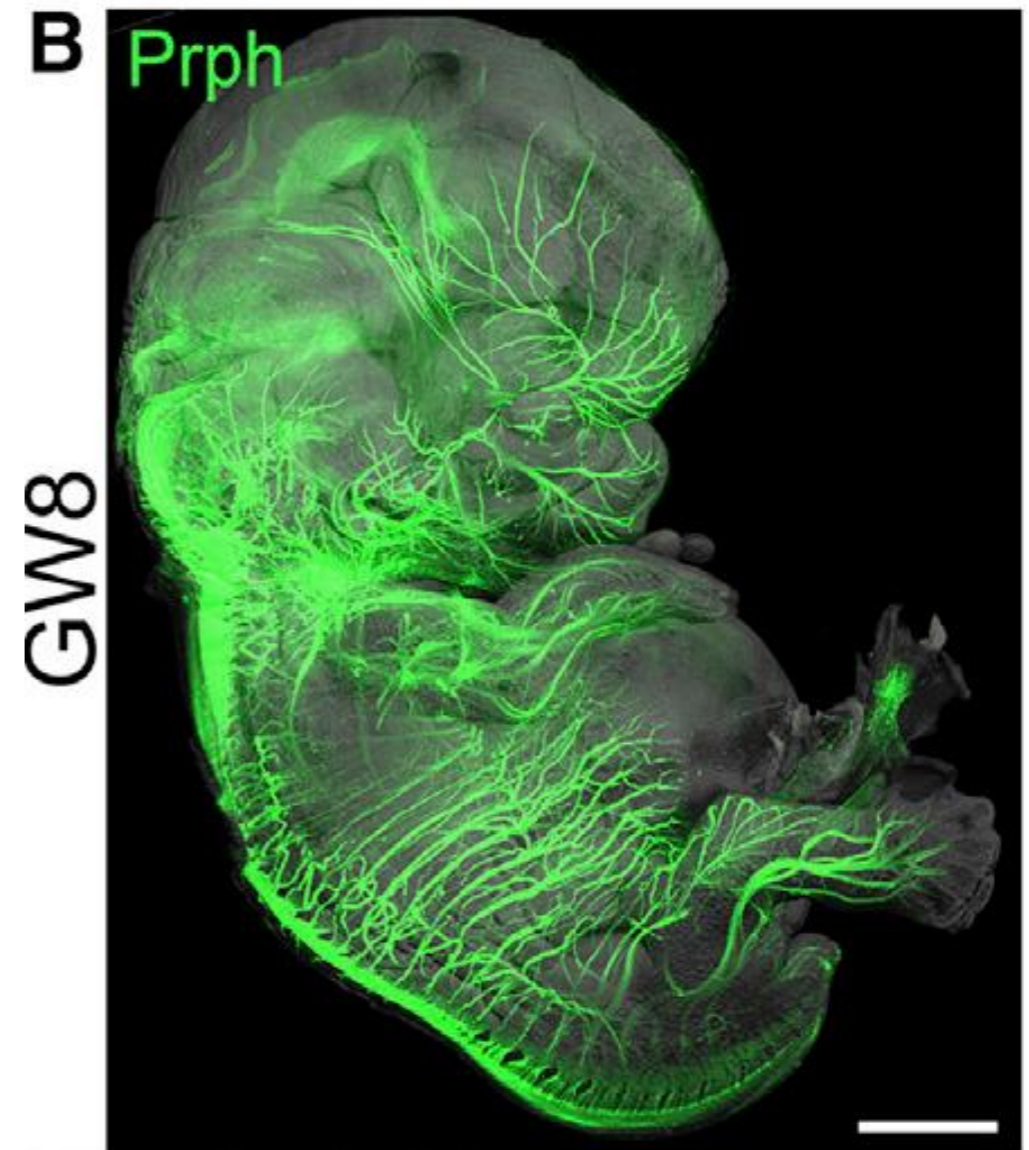
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Challenges and future directions

- Are the PSN not fully functionally mature? Can we improve that?
- Is **contact** with keratinocytes indispensable rather than soluble factors?
- Can we model neuropathic nociception in vitro? Lack of human models, poor translation from rodents, poor response to pharmacological treatment



Project Team

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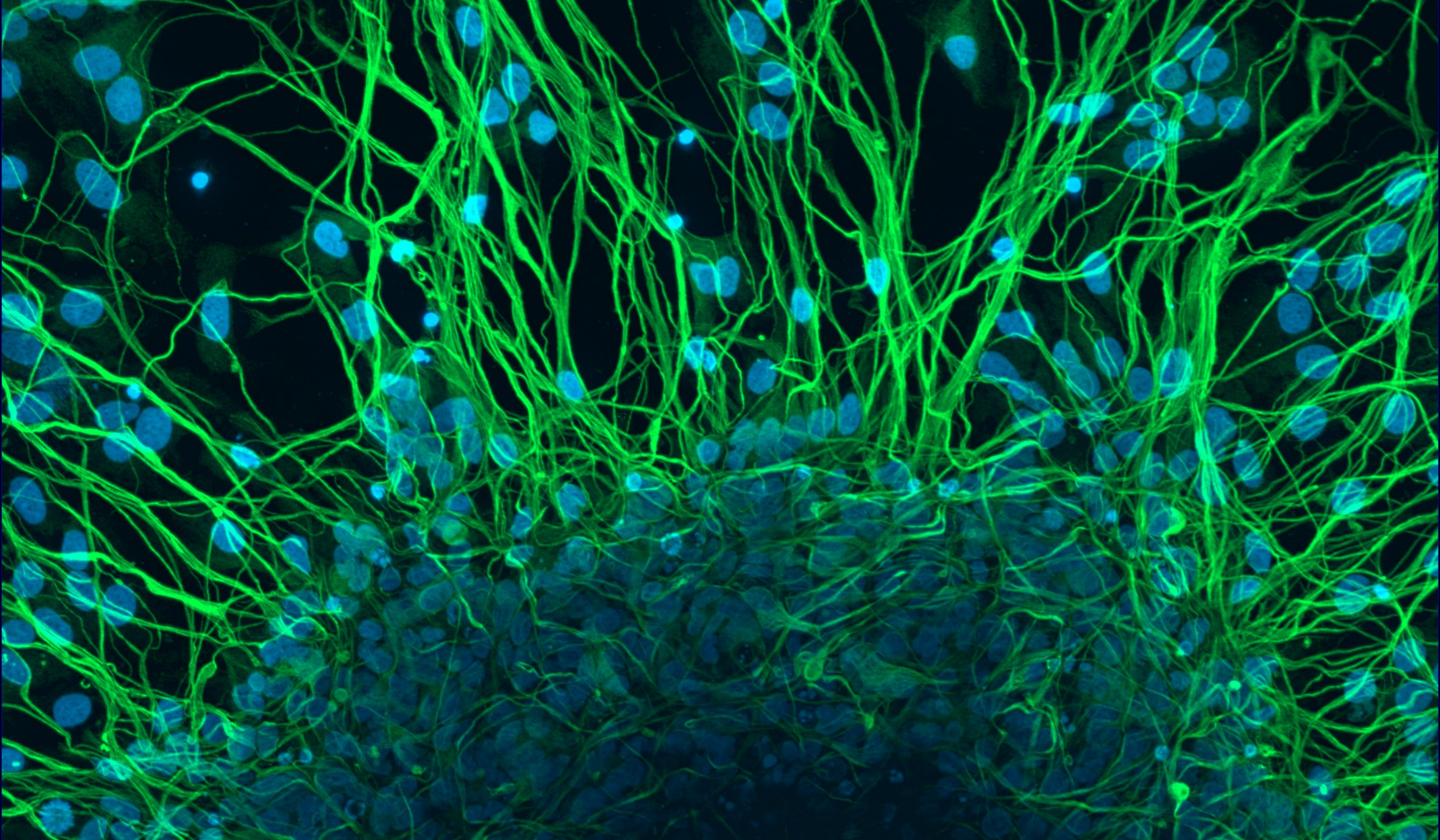
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Thank you!

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