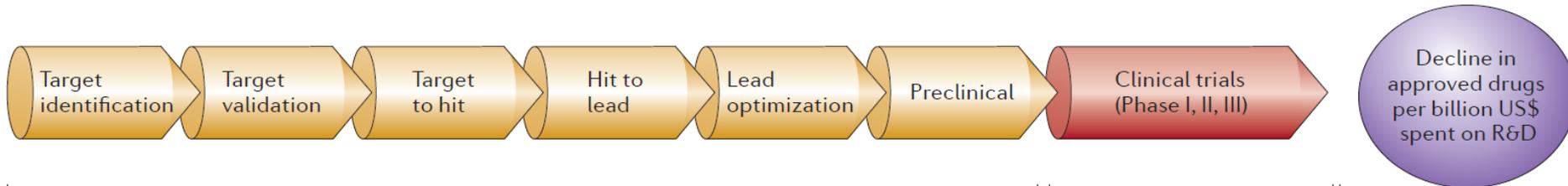
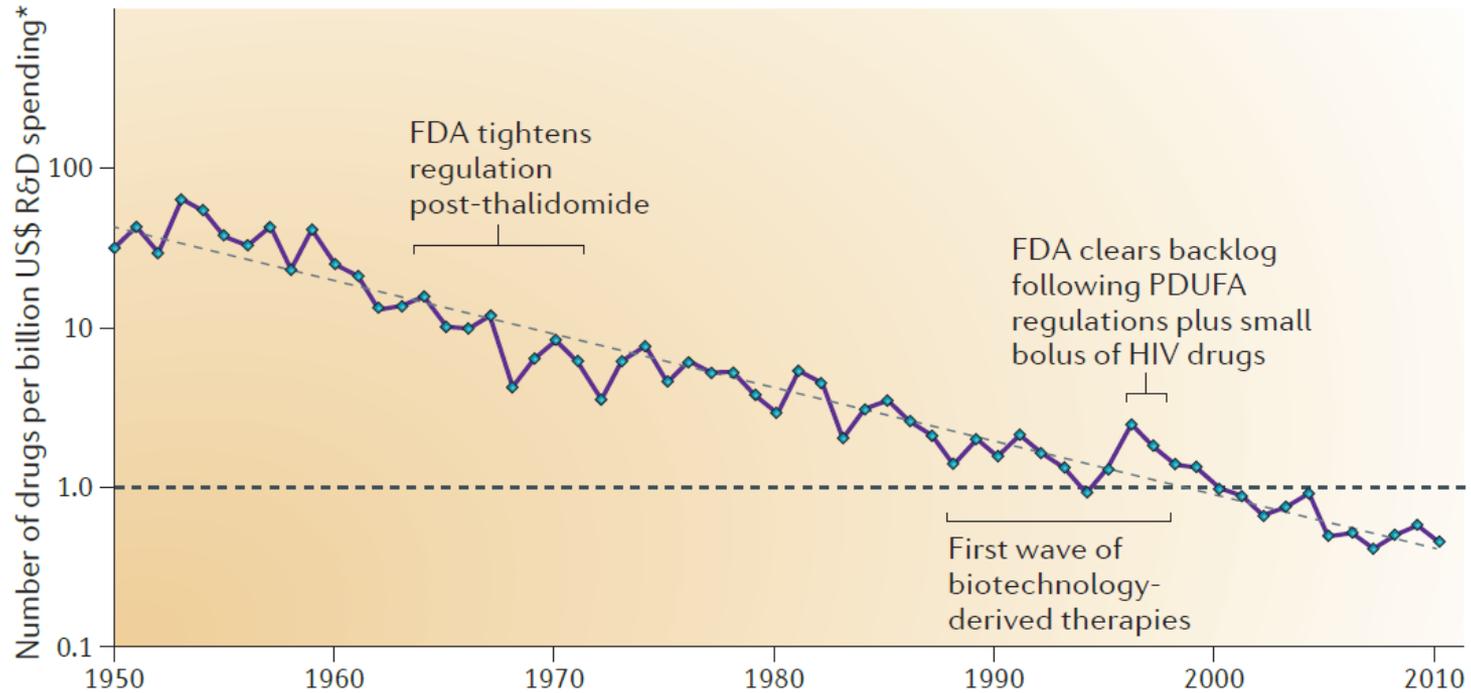


Pain in a Dish

Clifford J Woolf

Pharma Return on Investment

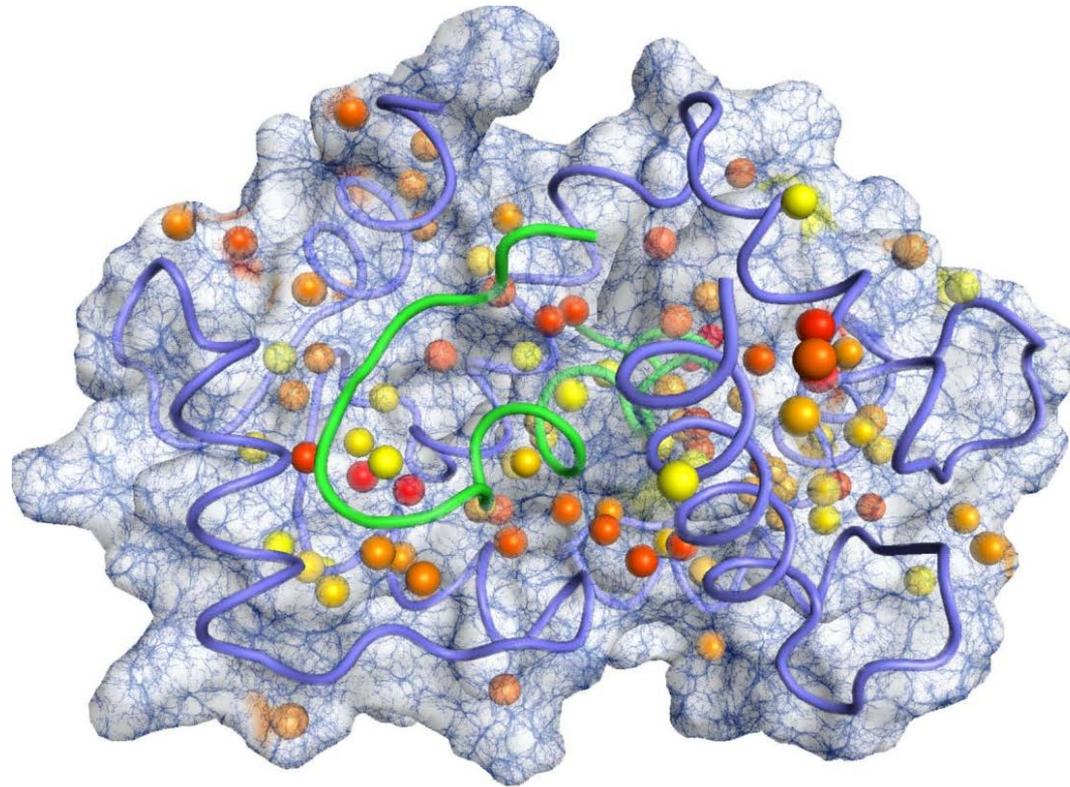
a Overall trend in R&D efficiency (inflation-adjusted)



Drug Target High Throughput Screening

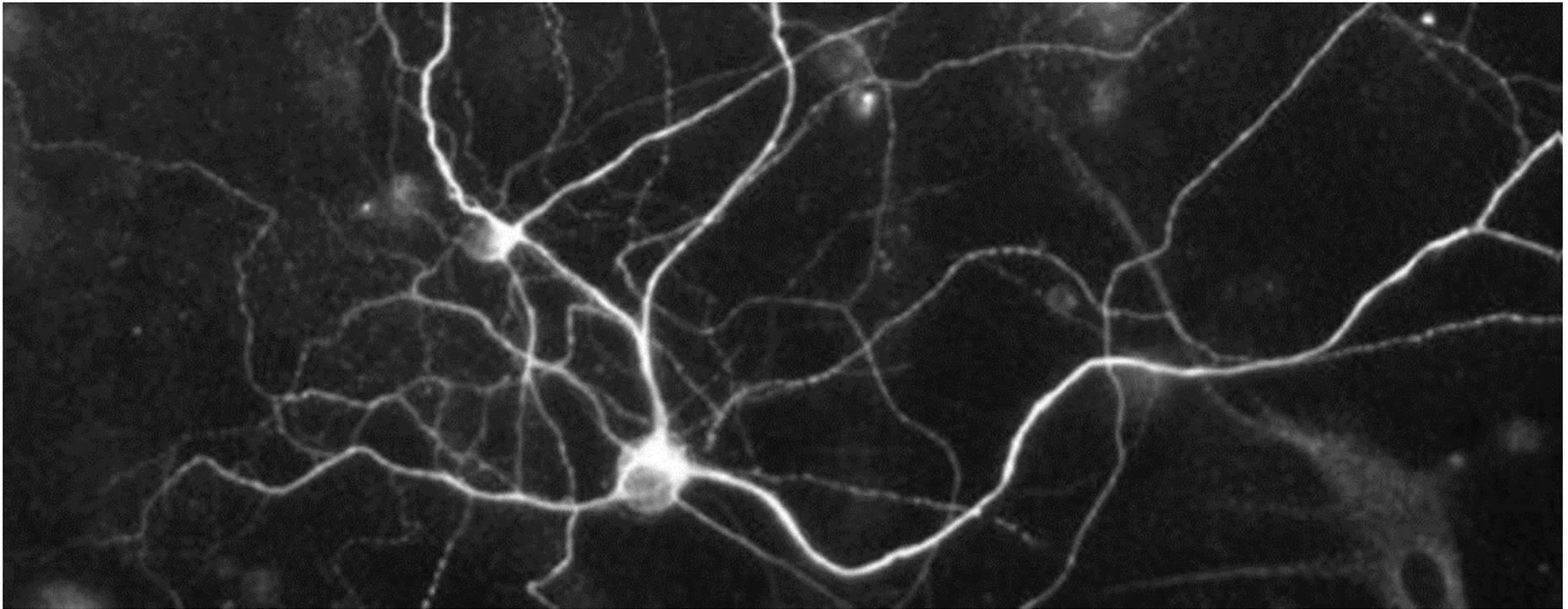


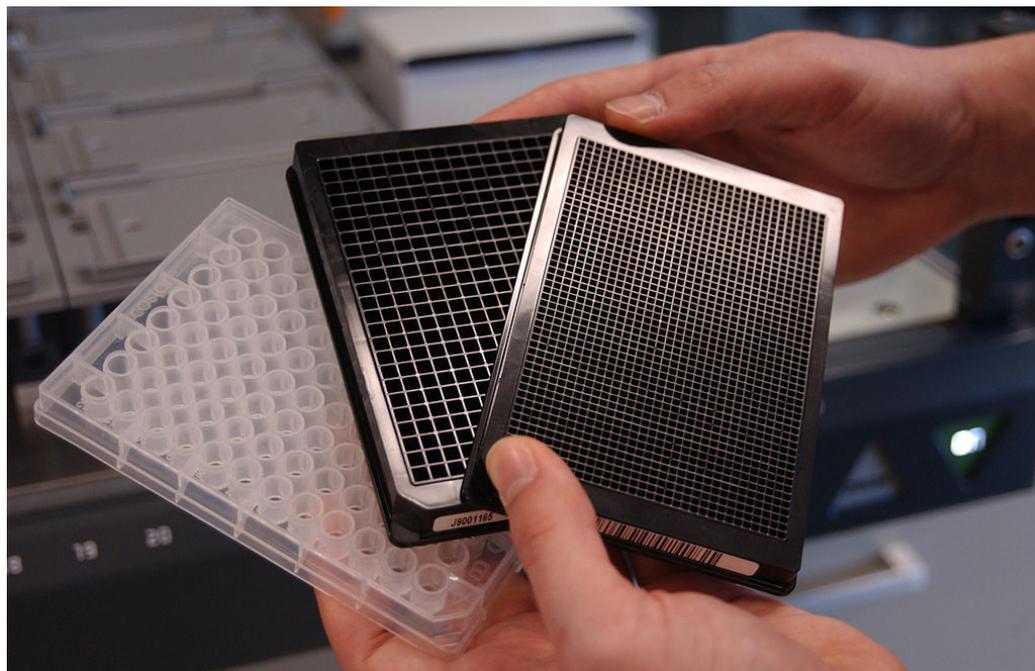
What Target?



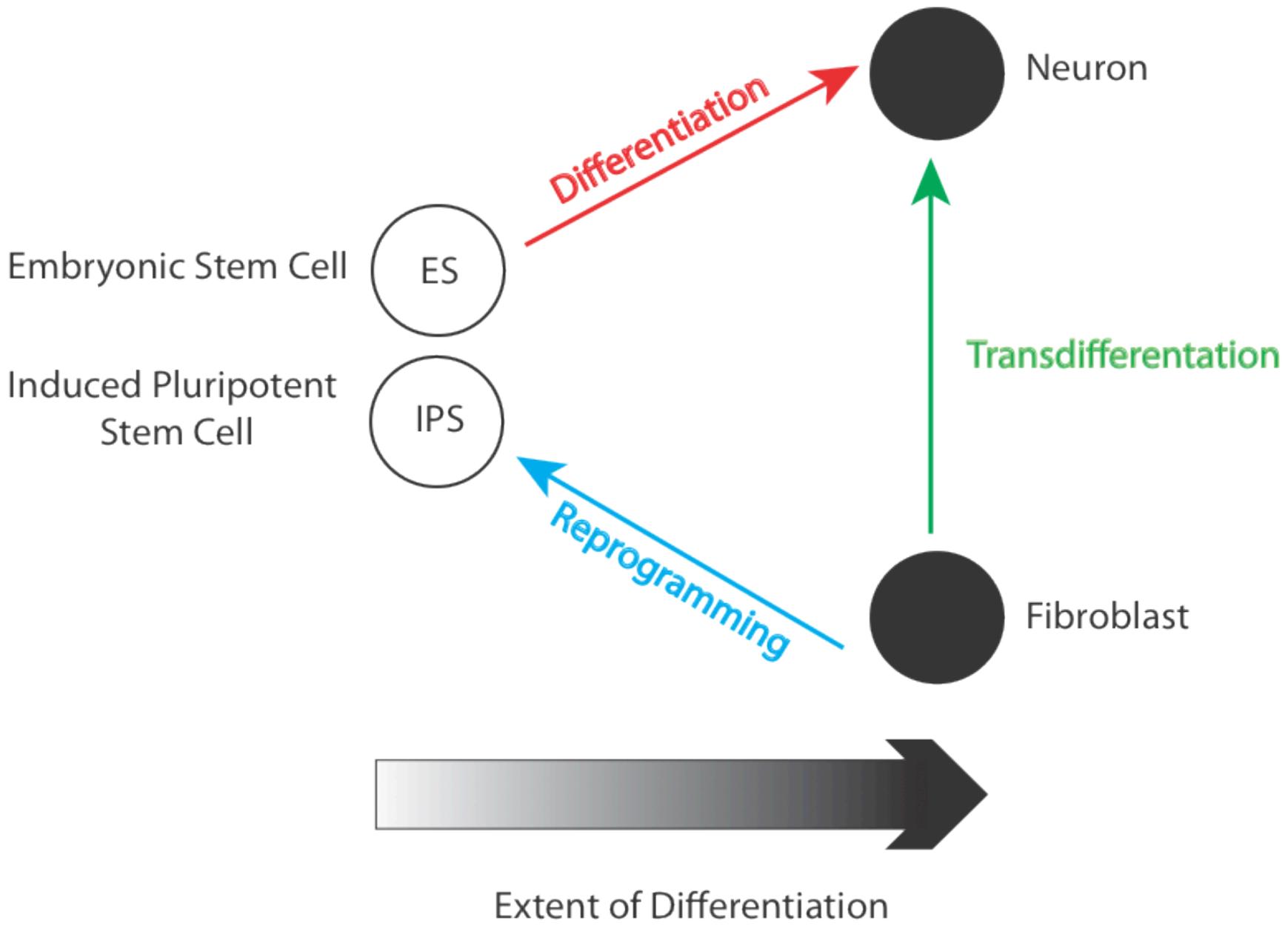
Heterologous expression in HEK or CHO cells
does not replicate
native receptor properties

Study human receptors in human neurons

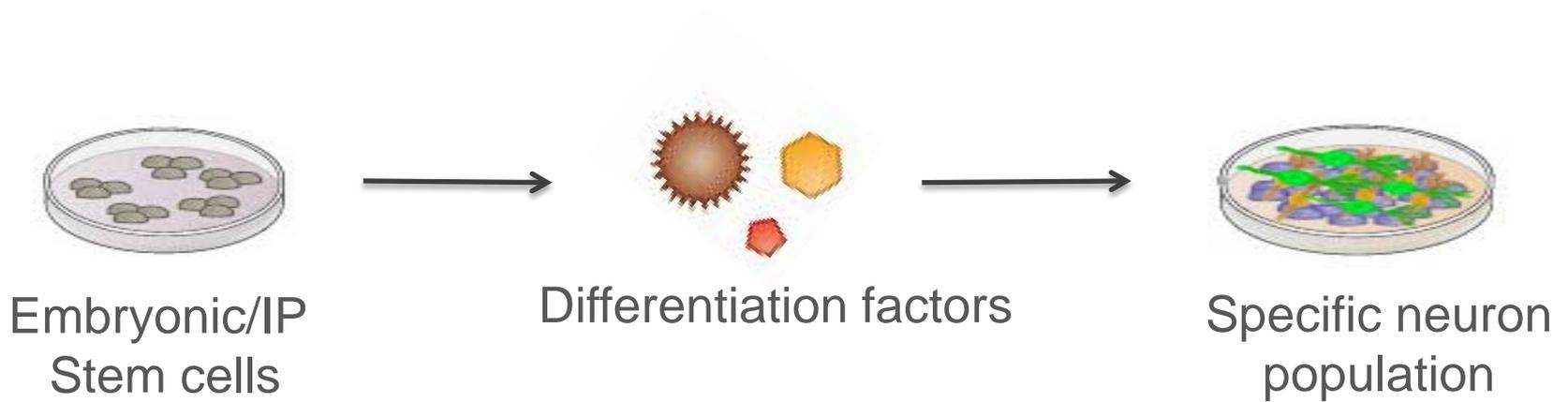




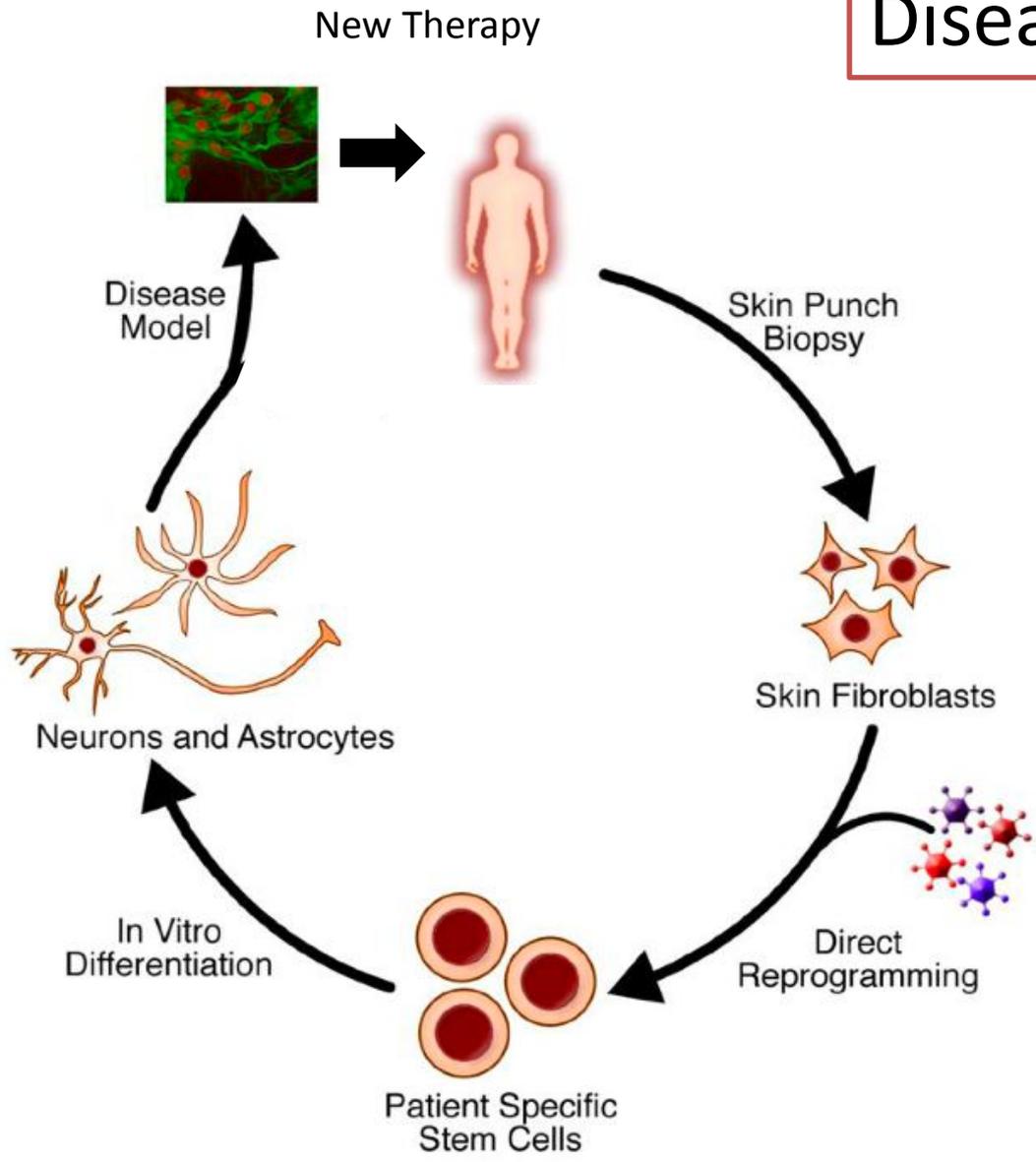
Phenotypic screen - disease in a dish



Directed human neuronal differentiation



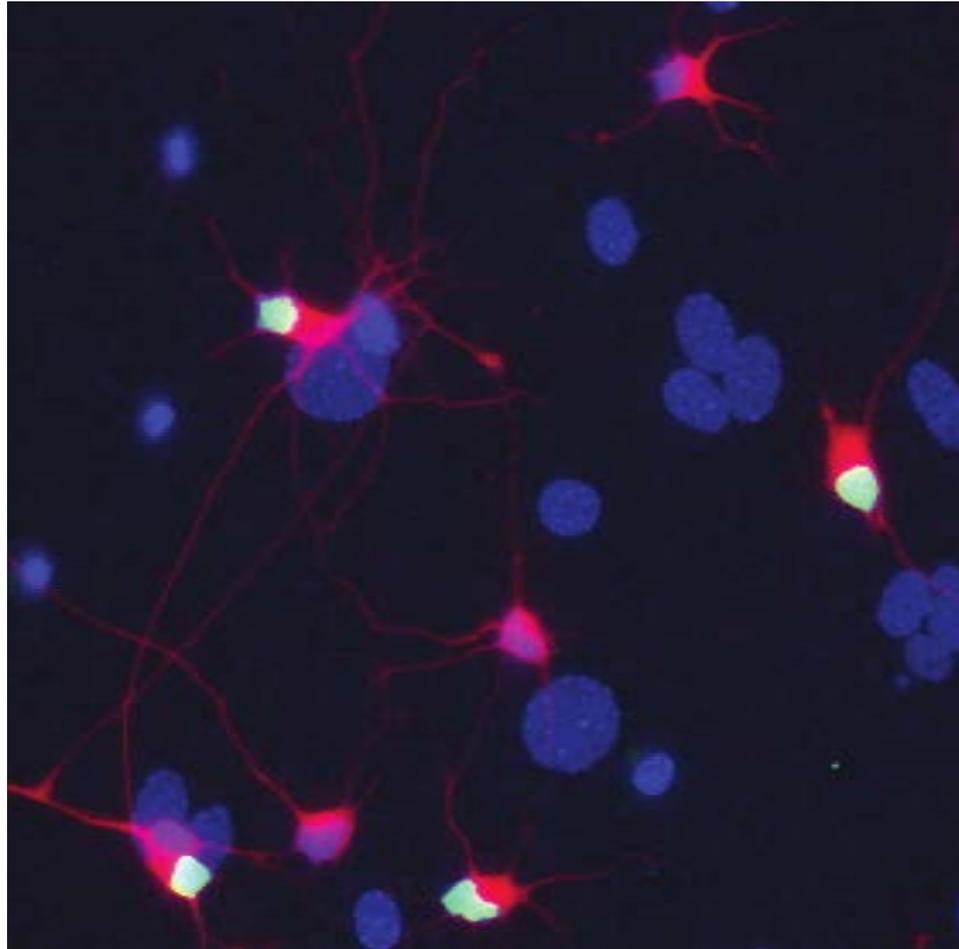
Disease Modeling



Neuronal specification in patient iPSC lines

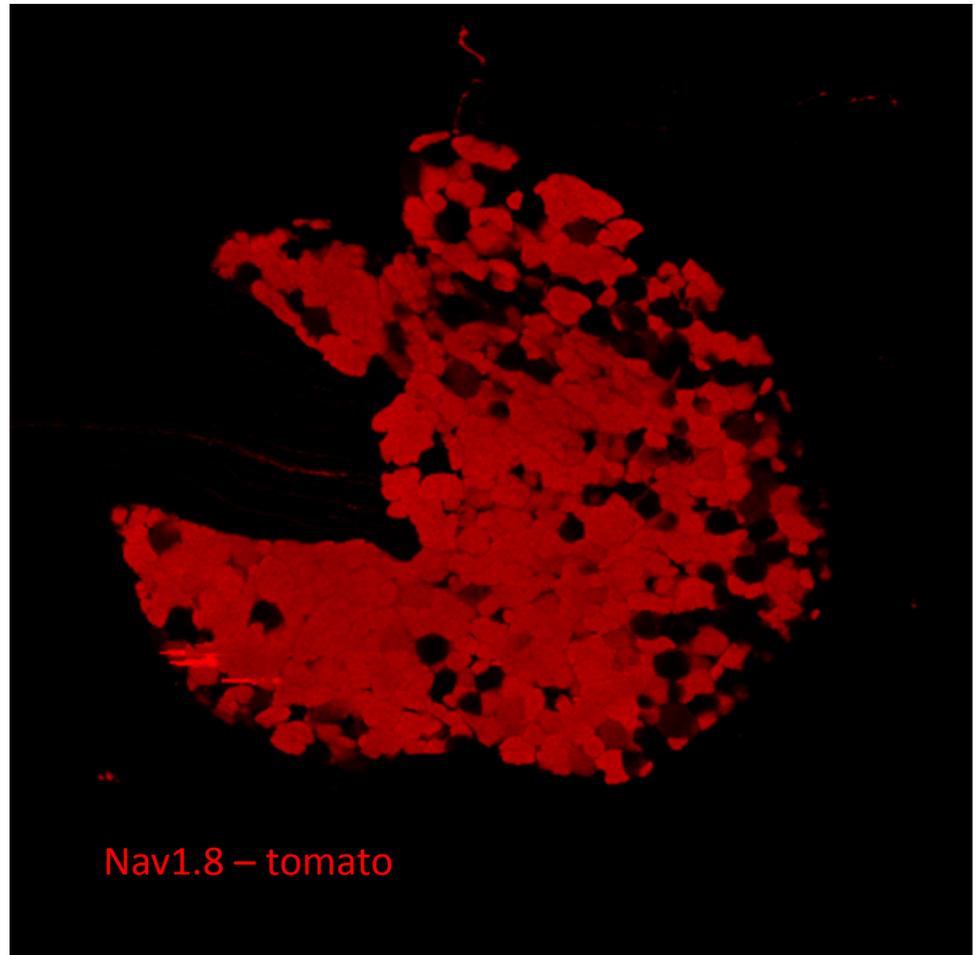
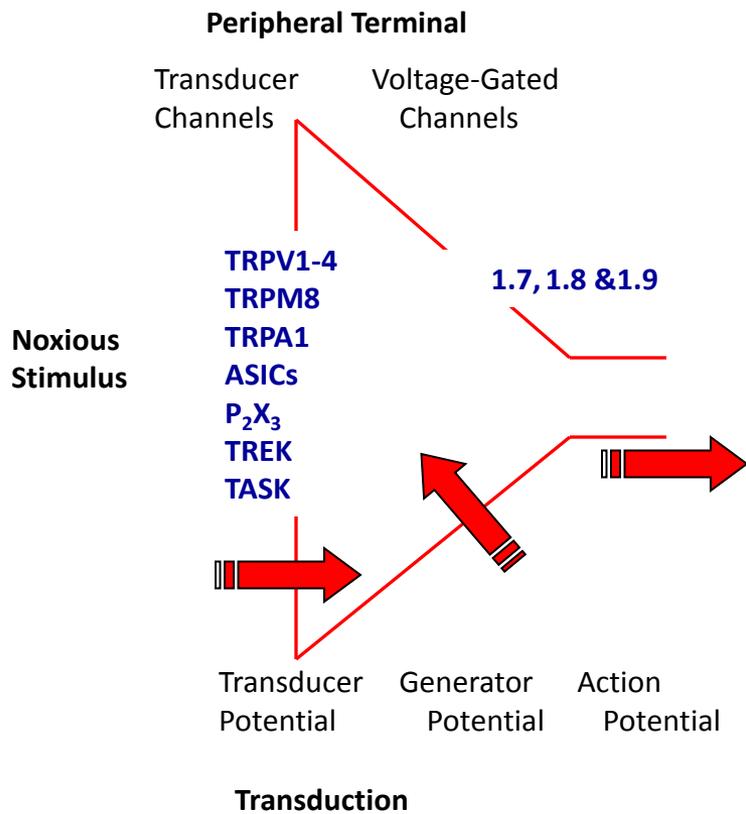
DNA ISL TUJ1

SOD1A4V 39b



What about pain?





A fluorescence micrograph showing a dense network of neurons. The cell bodies and processes are stained with a bright green fluorescent dye, creating a complex, interconnected web of lines and nodes against a dark background. The neurons vary in size and shape, with some having prominent, rounded cell bodies and others appearing as smaller, more delicate structures.

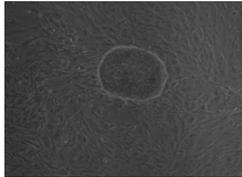
Screen for analgesic efficacy in human nociceptors

Study pain channelopathies in human neurons

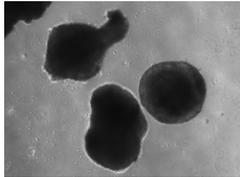
Determine risk of developing pain/neuropathy

Directed differentiation

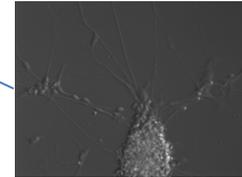
Undifferentiated hESC



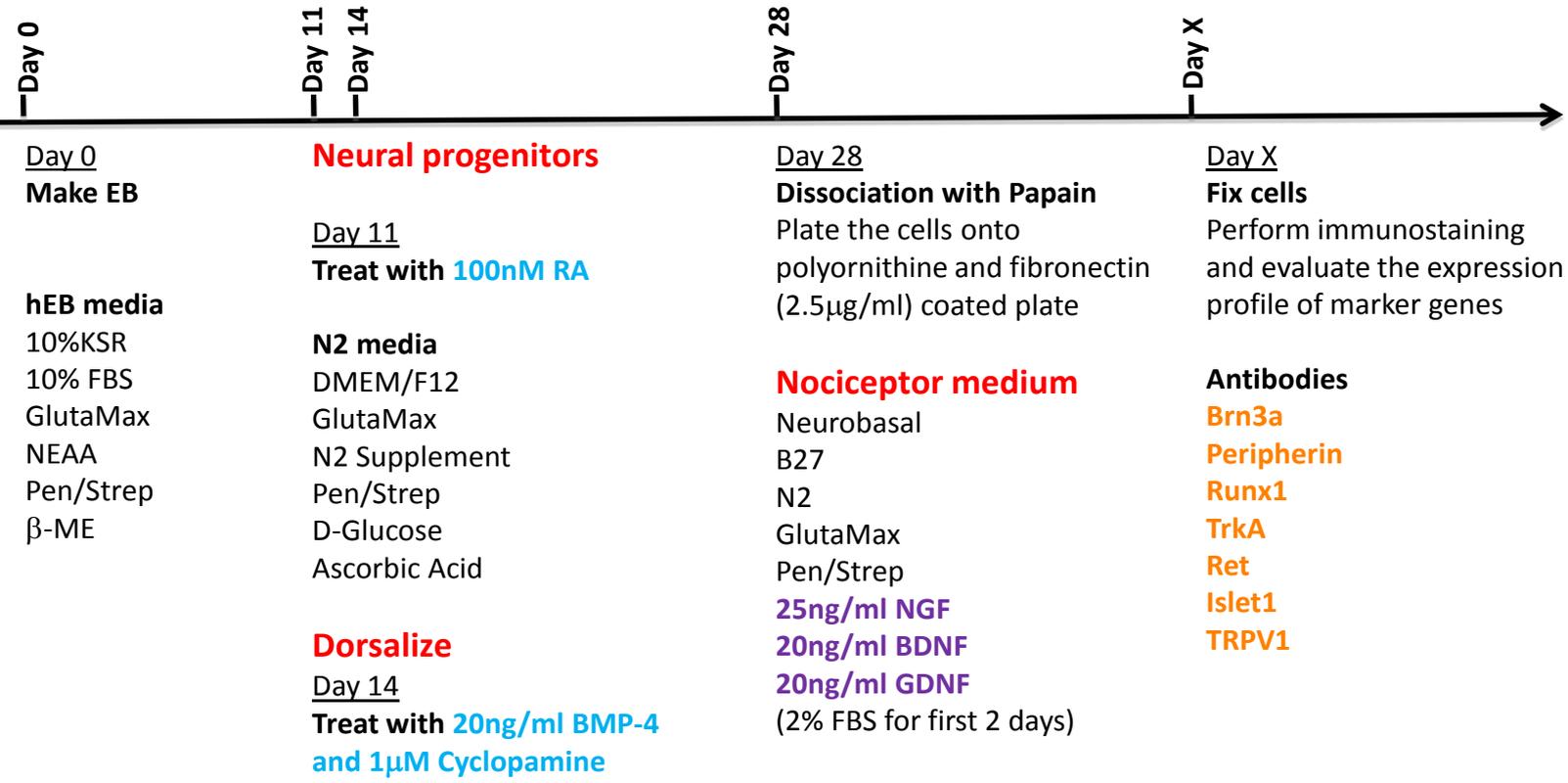
EB



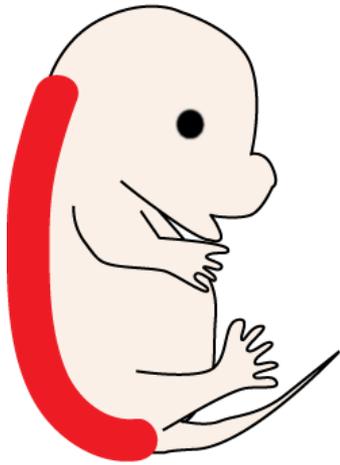
Neuron



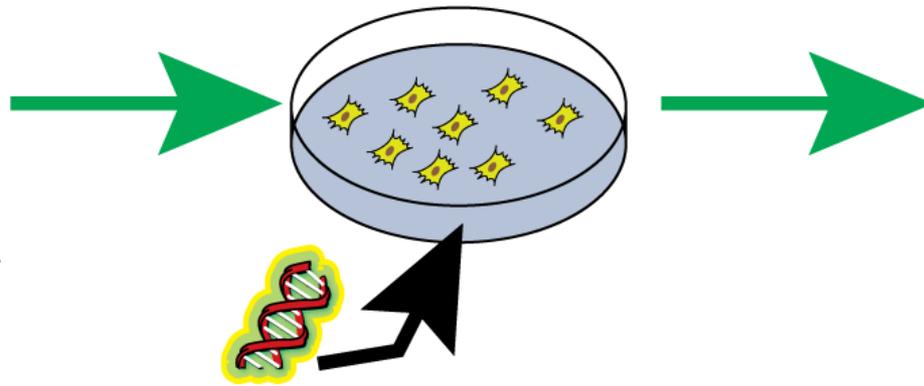
- HUES lines**
- HUES1
- HUES2
- HUES3
- HUES4
- HUES5
- HUES6
- HUES7
- HUES8
- HUES9
- HUES10
- HUES11
- HUES12
- HUES13
- HUES14
- HUES15
- HUES16
- HUES17



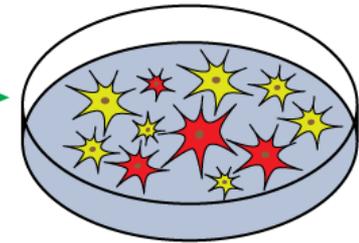
Transdifferentiation



Isolate MEFs from NaV1.8::tomato mice.

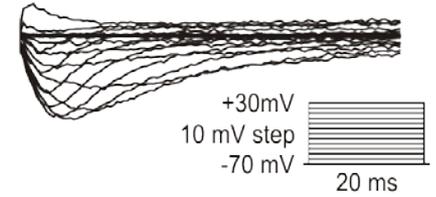
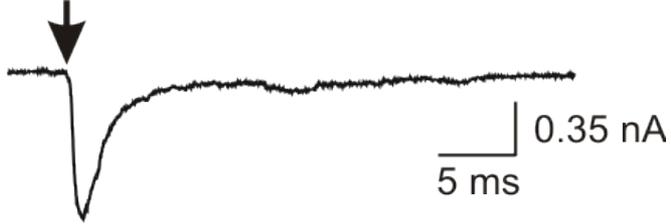


Transduce MEFs with BAM and nociceptor factors.



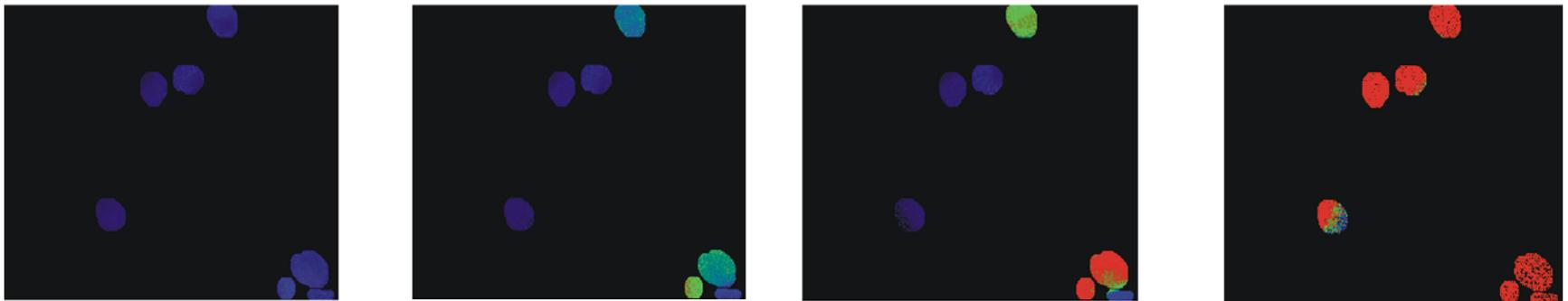
Detect neuronal morphology and activation of tomato reporter.

Capsaicin



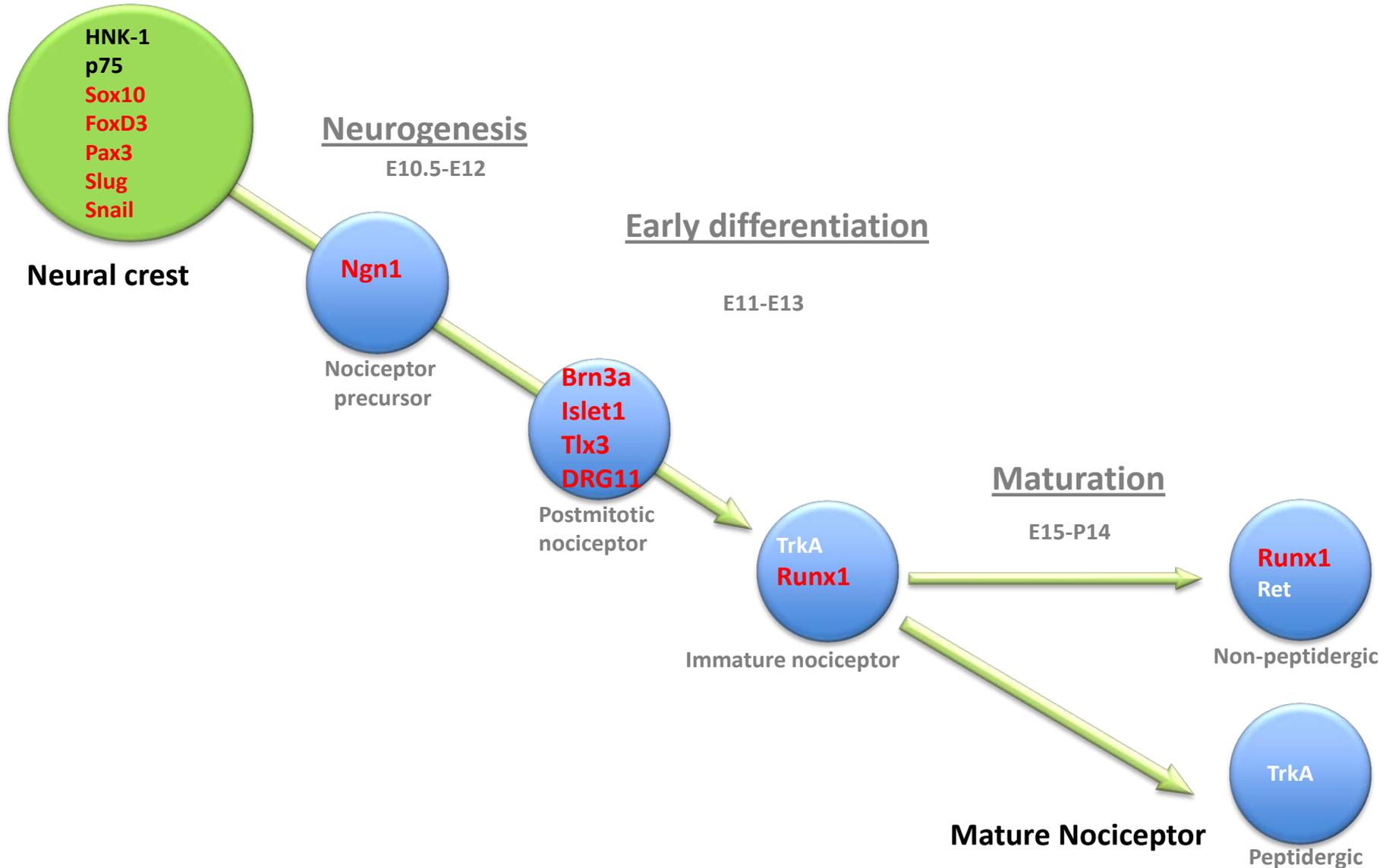
Voltage clamp

Phenotyping Nociceptors



Calcium Imaging

Which Transcription factors?





Transcriptional profiling at whole population and single cell levels reveals somatosensory neuron molecular diversity

Isaac M Chiu^{1,2,6*}, Lee B Barrett^{1,2}, Erika K Williams³, David E Strohlic³, Seungkyu Lee^{1,2}, Andy D Weyer⁴, Shan Lou⁵, Gregory Bryman^{1,2}, David P Roberson^{1,2}, Nader Ghasemlou^{1,2}, Cara Piccoli^{1,2}, Ezgi Ahat^{1,2}, Victor Wang^{1,2}, Enrique J Cobos^{1,2,7}, Cheryl L Stucky⁴, Qiufu Ma⁵, Stephen D Liberles³, Clifford J Woolf^{1,2*}

Three years



Five factors & four weeks...

Plate
Split
Plat-E
Cells

Add
virus to
MEF
culture

Media
&
Growth
Factors

D 1-3

D 5

Fibroblasts

D 7

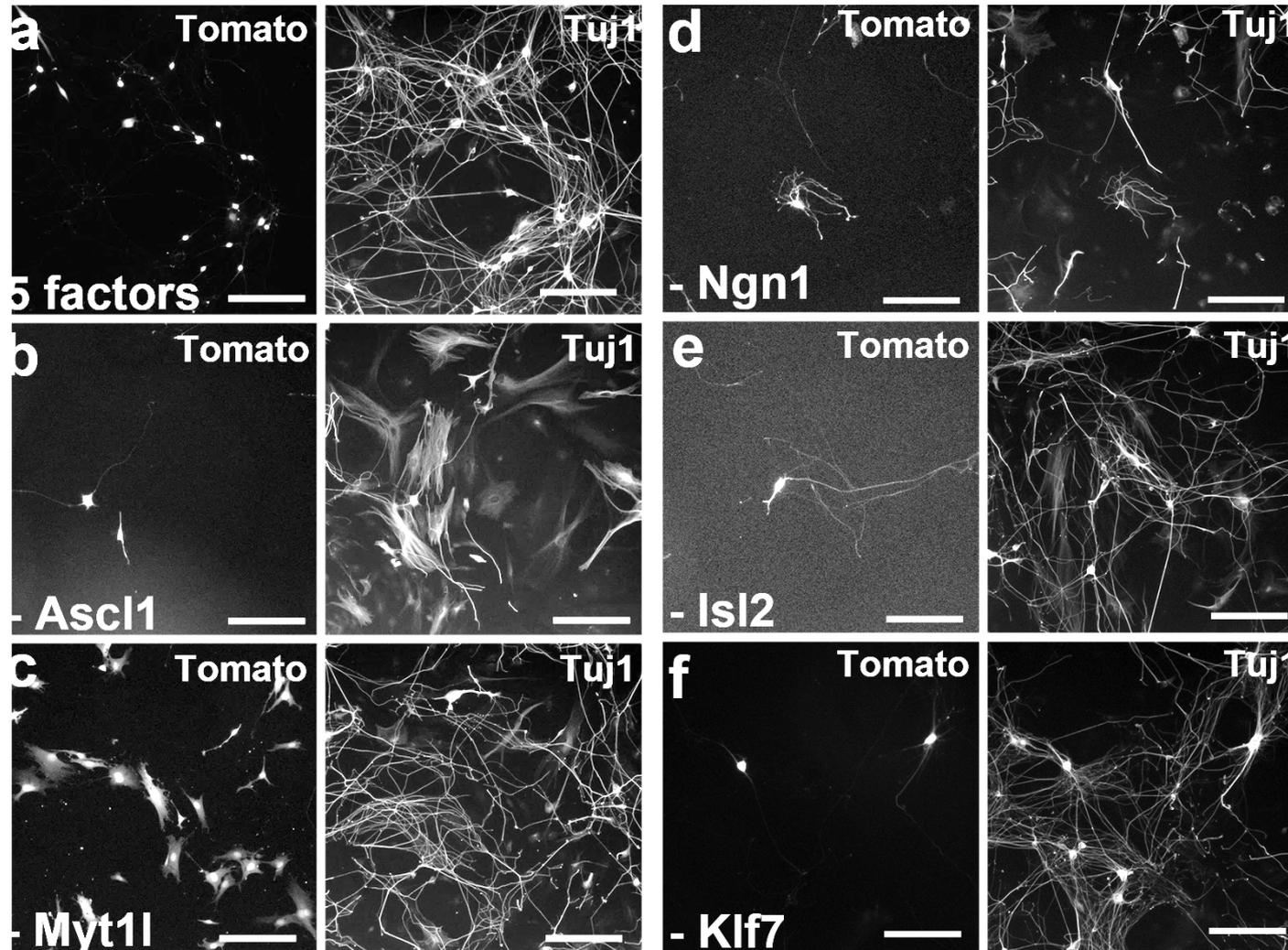
D 9

D 10

Neurons

Co-
culture
with
Glia

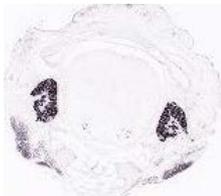
A combination of 5 factors results in efficient production of tomato-positive neurons



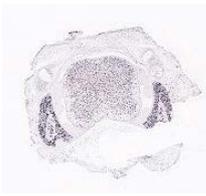
What are the 5 factors?

<u>Gene</u>	<u>Source</u>	<u>Family</u>	<u>Role in Reprogramming/ Sensory System</u>
Ascl1 (Achaete-Scute complex homolog1)	Lit	Basic helix-loop-helix/ achaete-scute	Neuronal lineage reprogramming/ neuronal commitment
Isl2 (Insulin gene enhancer protein2)	Exp, BioGPS	Homeo-domain/ LIM region	Unknown *
Klf7 (Kruppel-like factor 7)	Lit, Exp, BioGPS	Zinc-finger/Krueppel like	TrkA maintenance
Myt11 (myelin TS factor1)	Lit	Zinc-finger	Neuronal lineage reprogramming
Ngn1 (Neurogenin1)	Lit	Basic helix-loop-helix	TrkA and subsequent TrpV1 expression

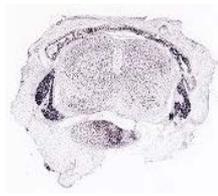
Isl2



Myt1



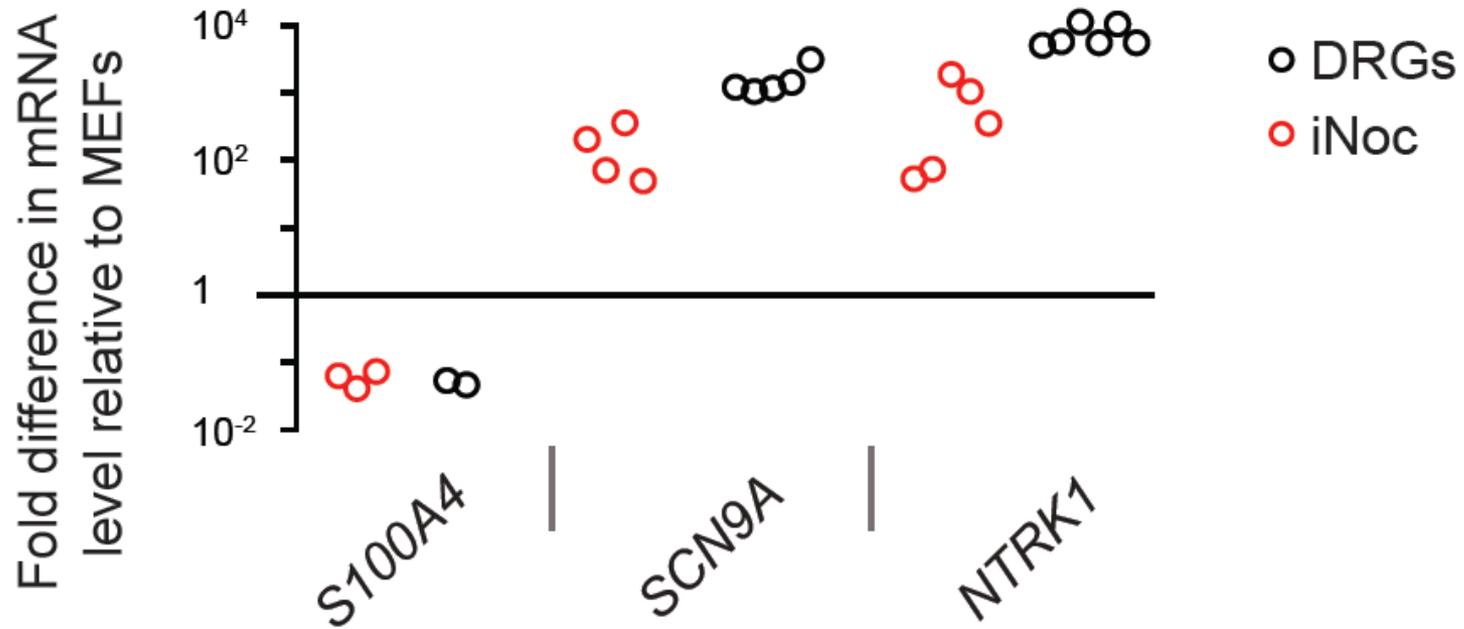
Klf7



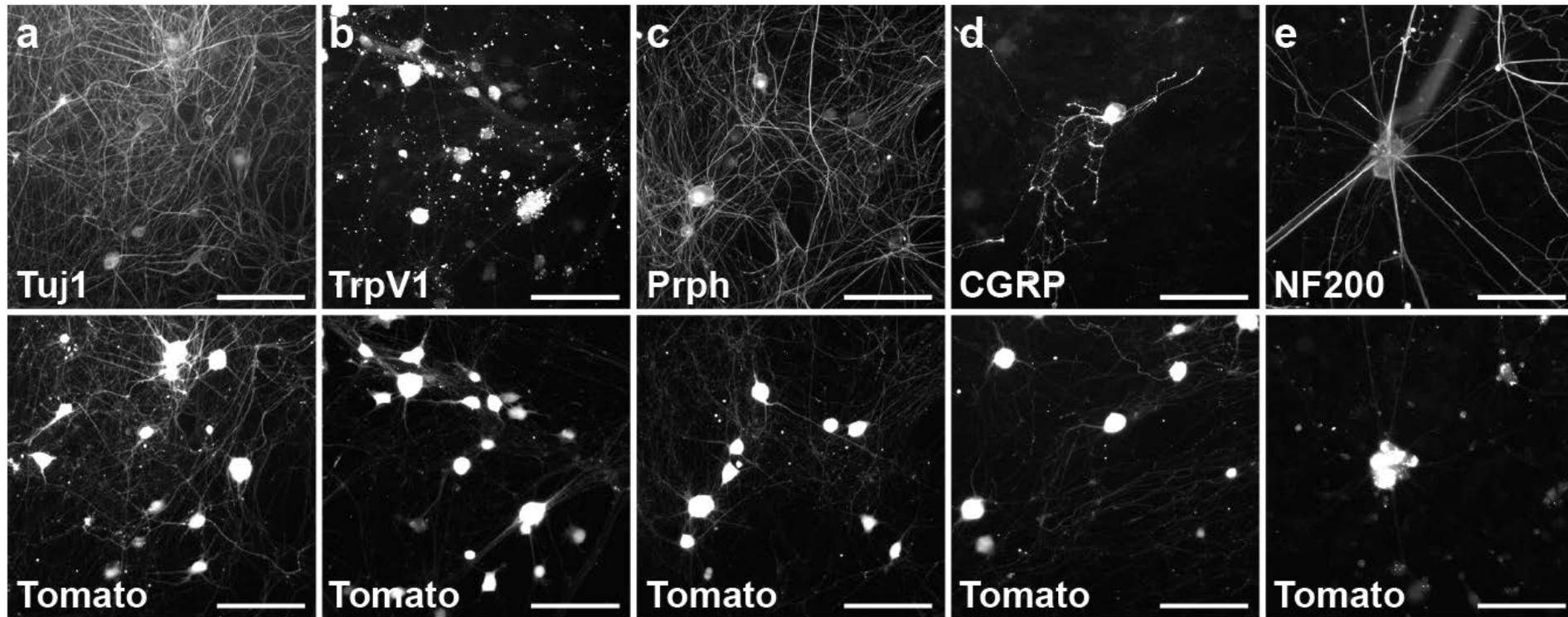
Allen Brain Atlas

*motor neuron identity, development and placement

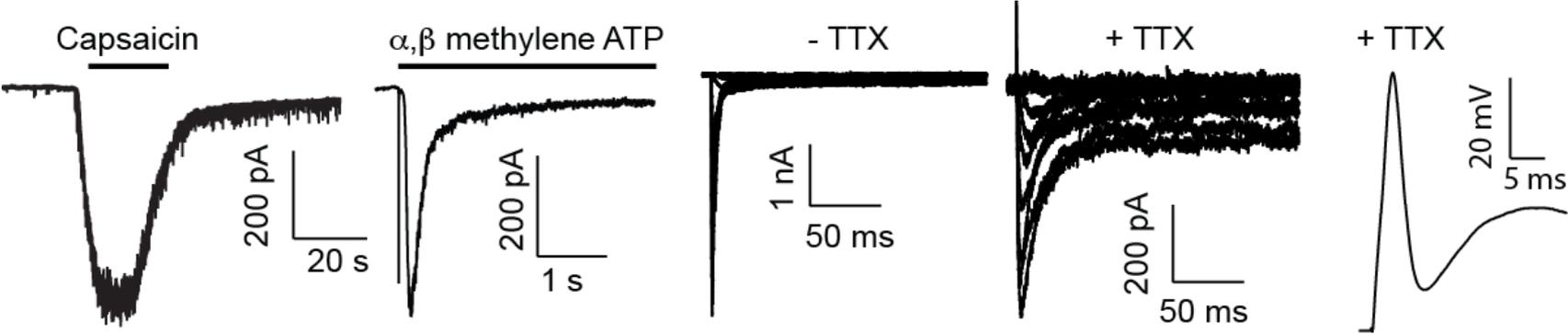
Expression of Nociceptor-Specific mRNAs



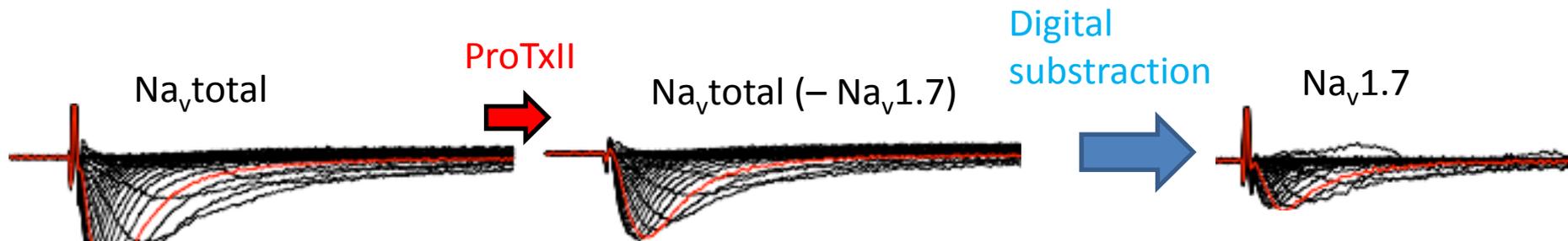
Molecular characterization of induced neurons



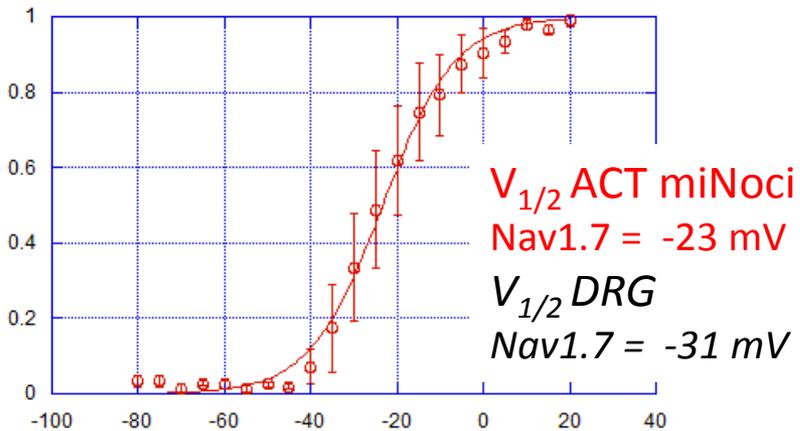
Electrophysiology of Induced Nociceptors



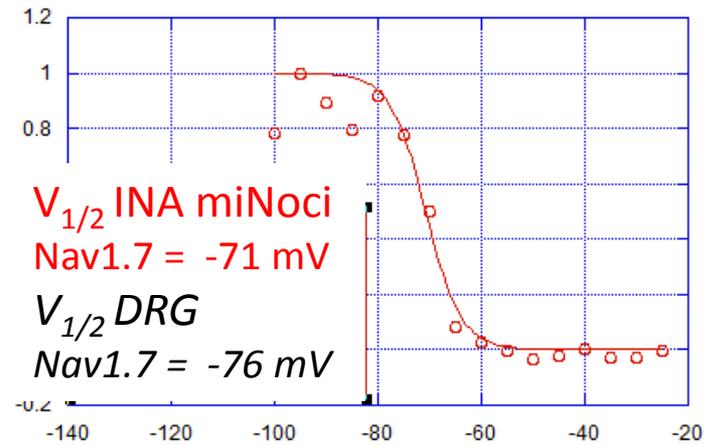
Nav1.7 current in miNoci → ProTxII



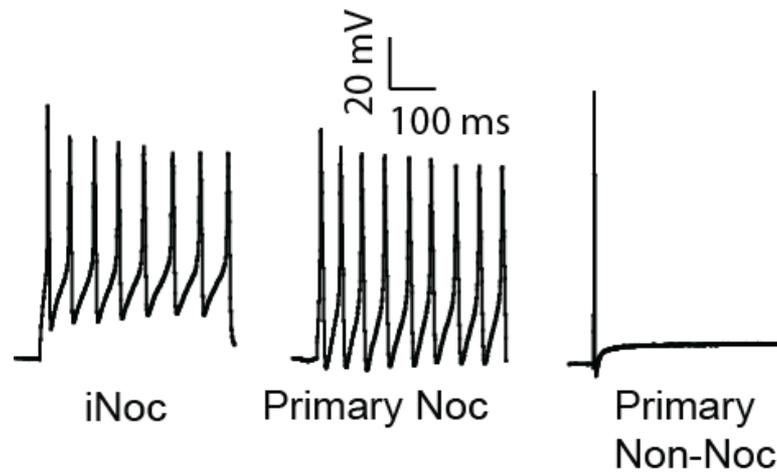
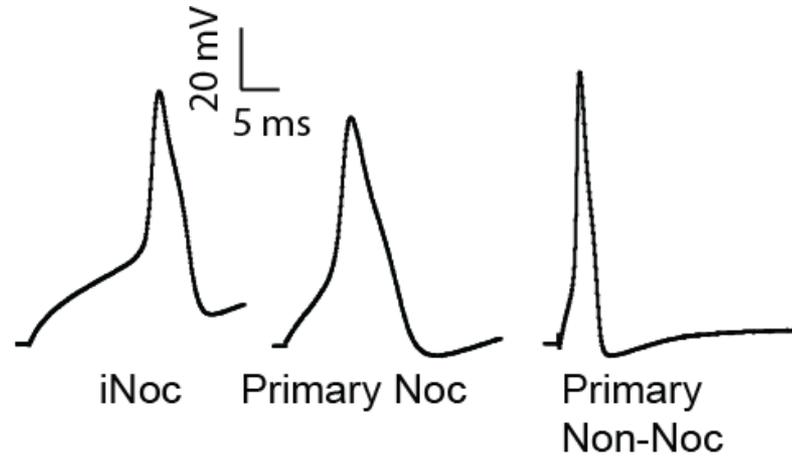
Data 14



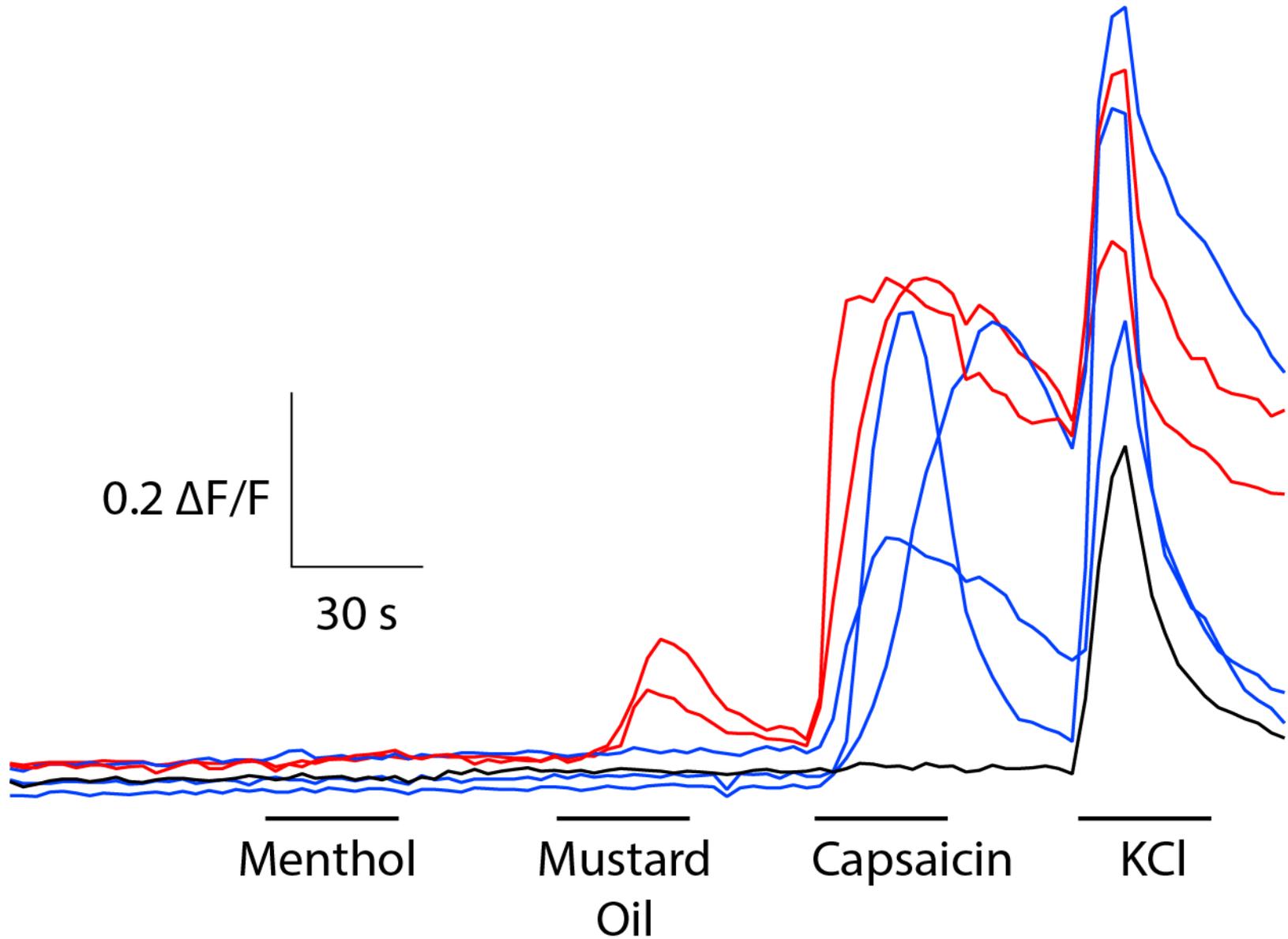
Data 8



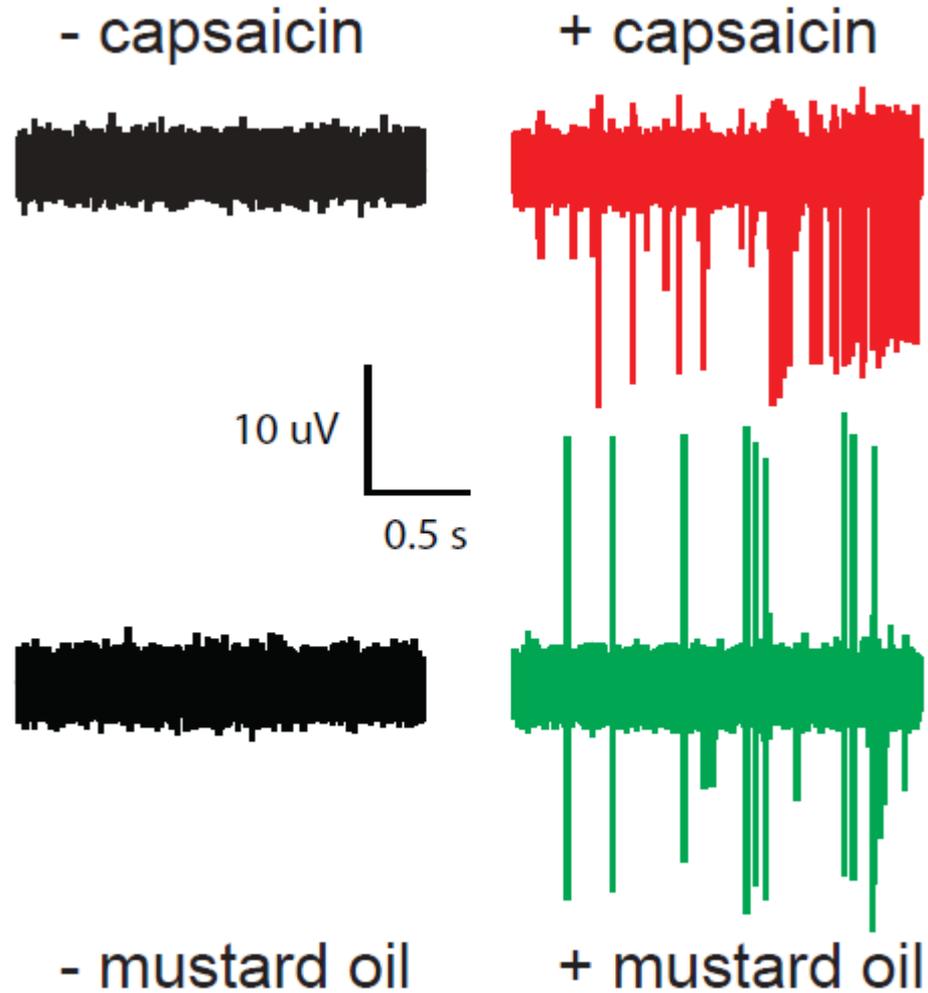
Action Potential and Firing Properties



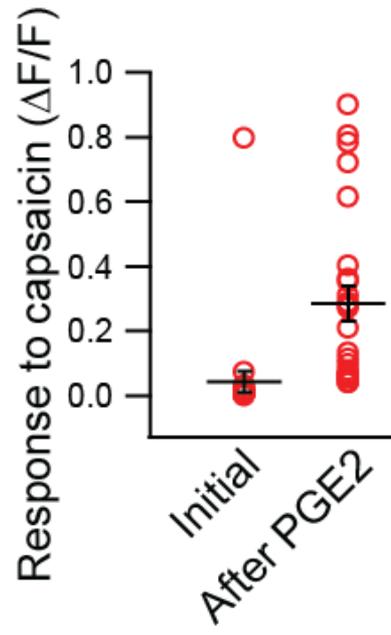
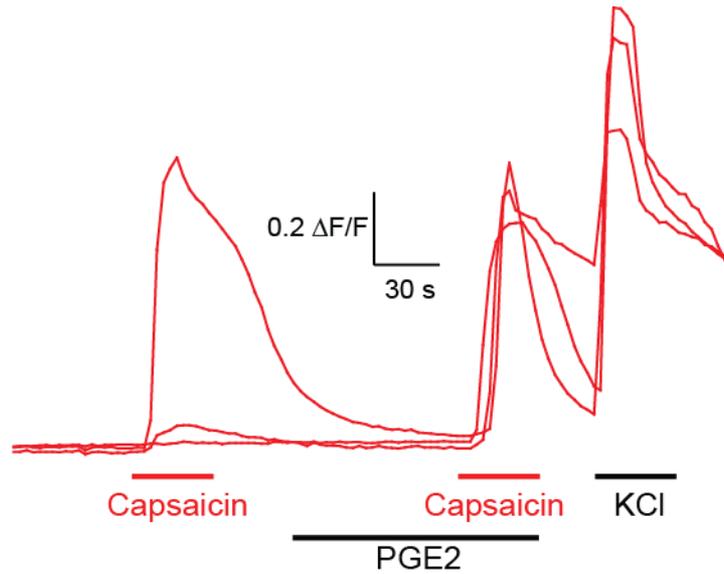
Functional Response to Trp Agonists



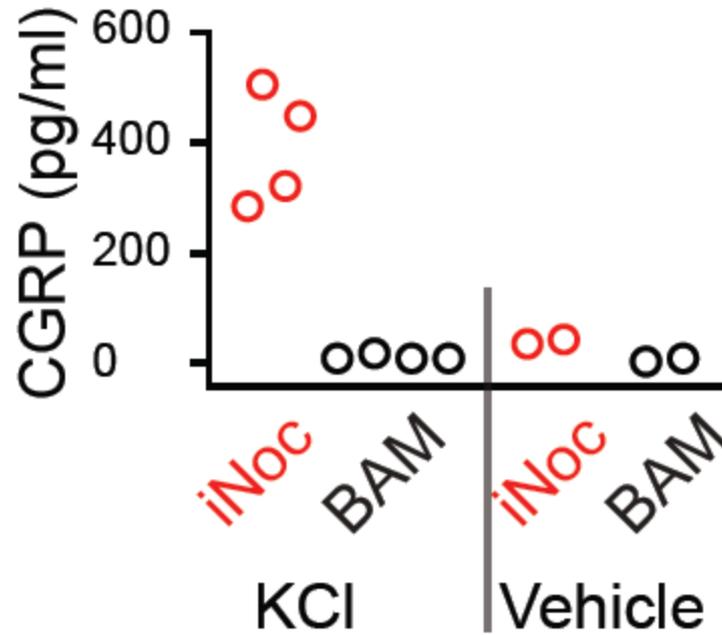
Induced Nociceptors Fire in Response to Trp Agonists

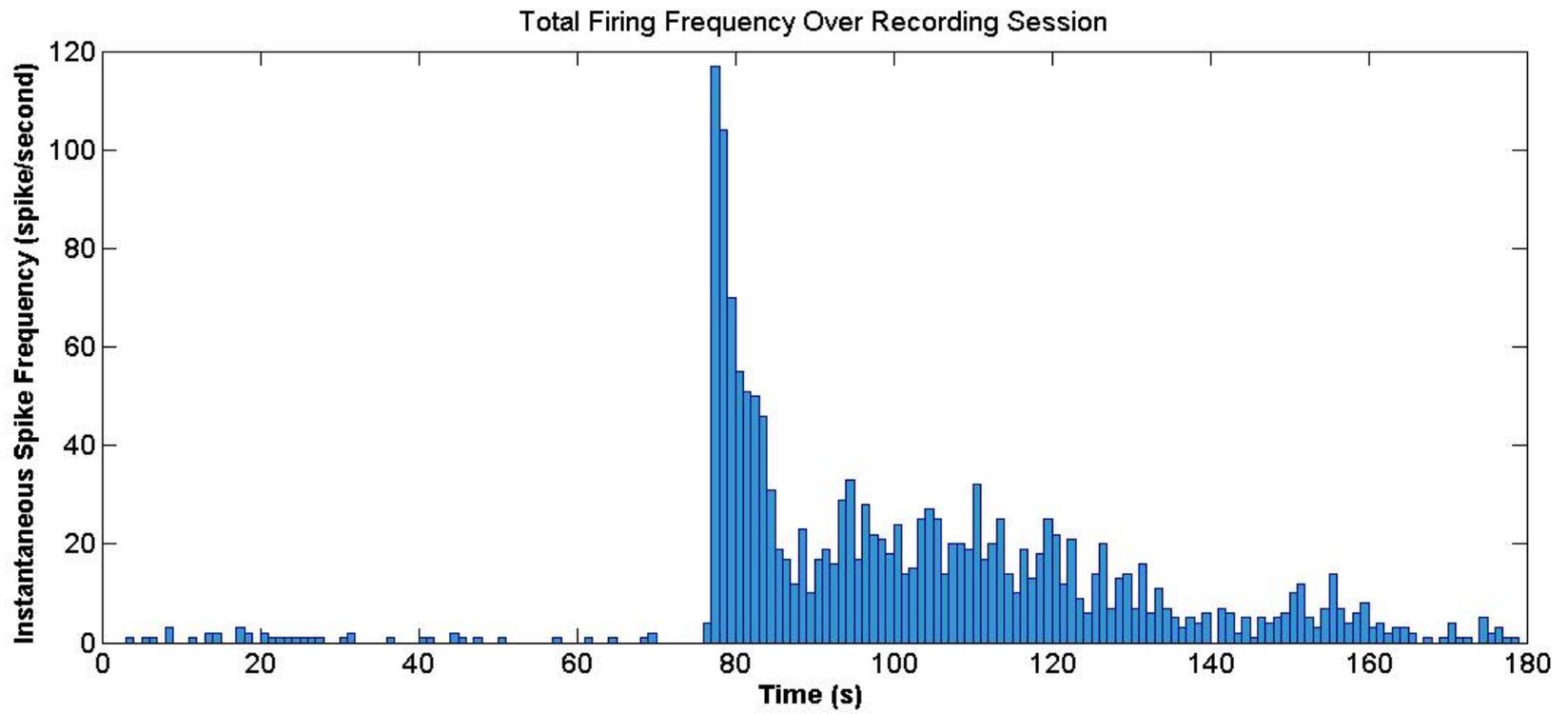


Sensitization by Inflammatory Mediators



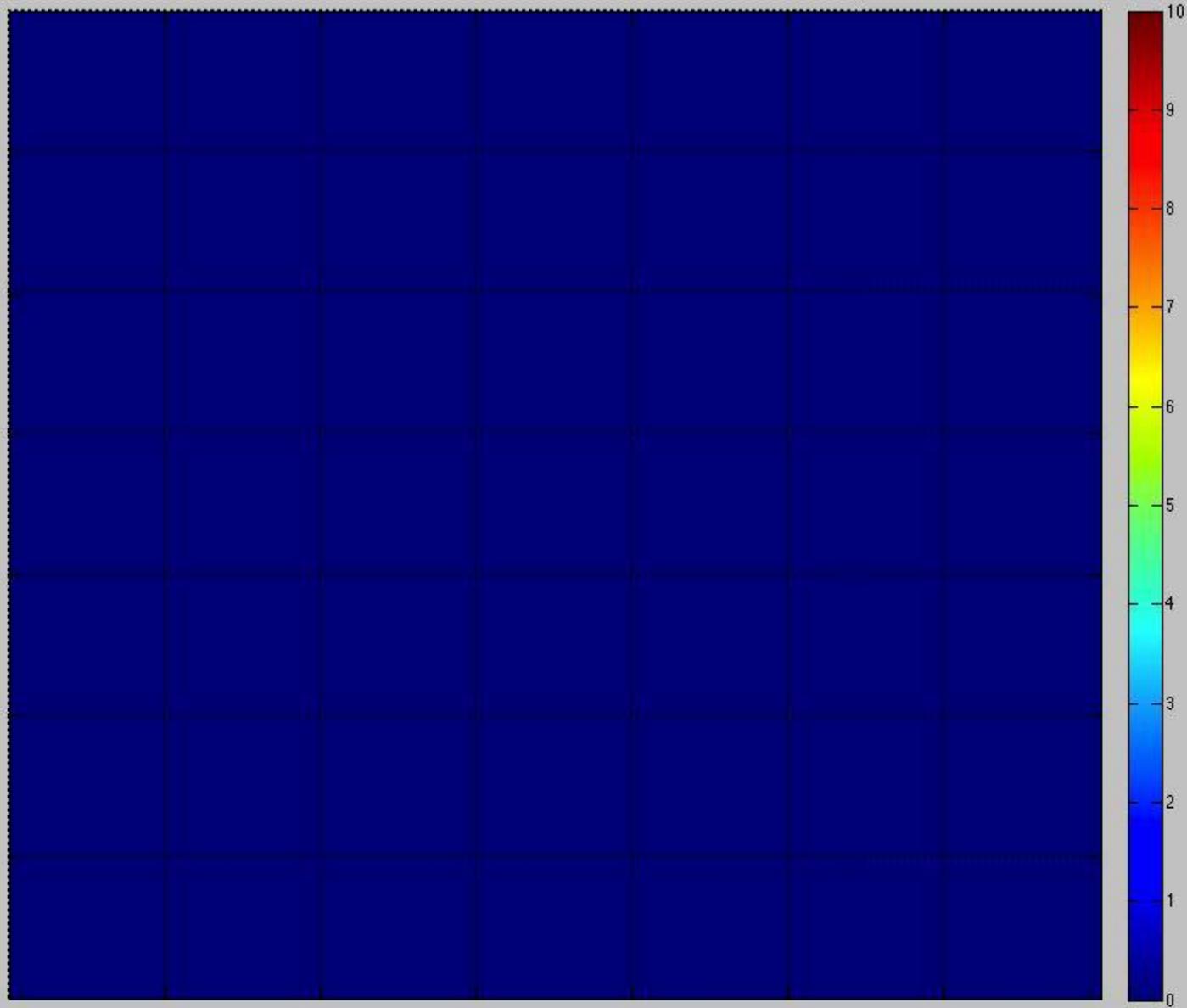
Transmitter release



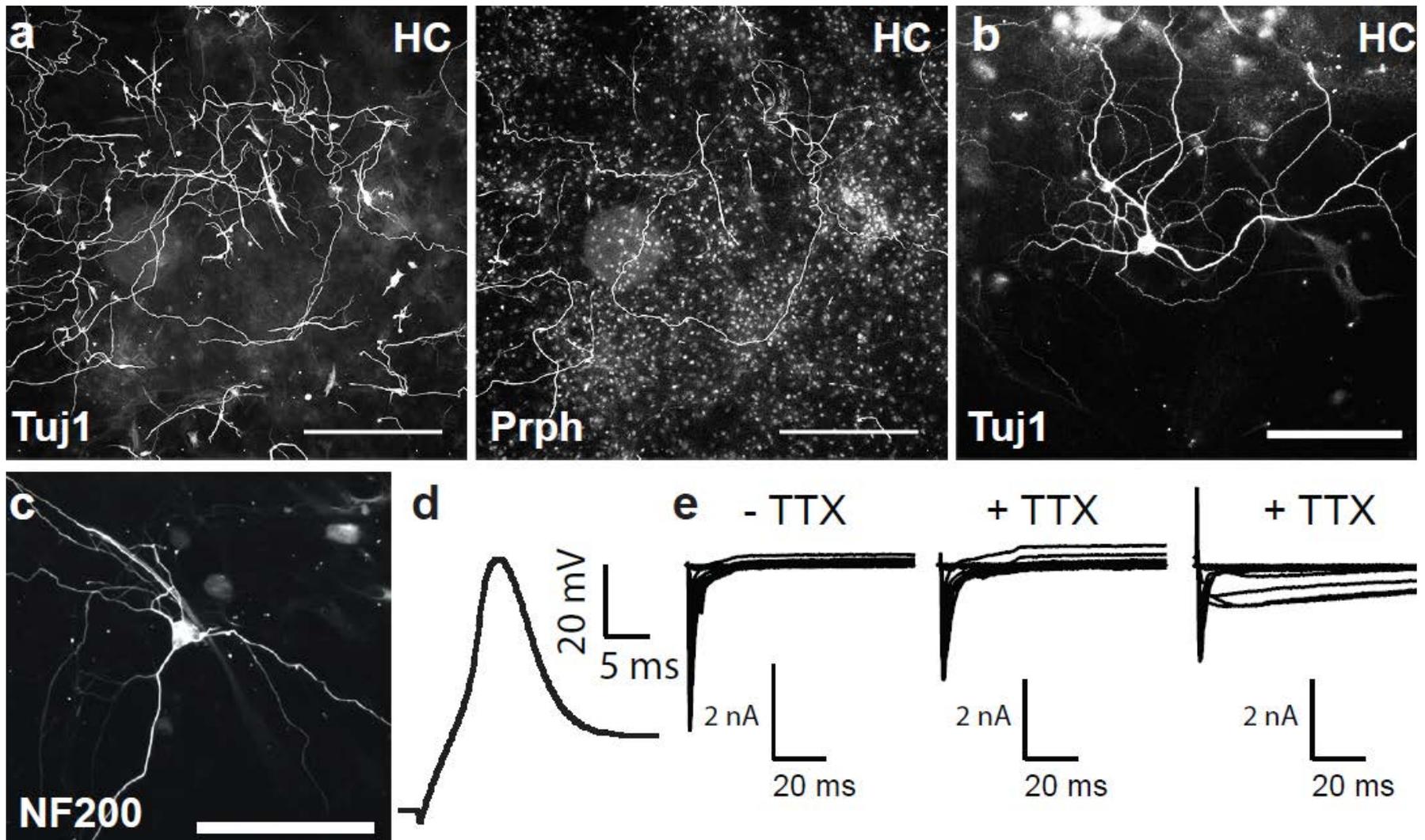


1 μ m capsaicin

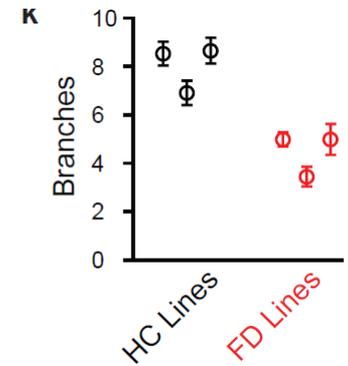
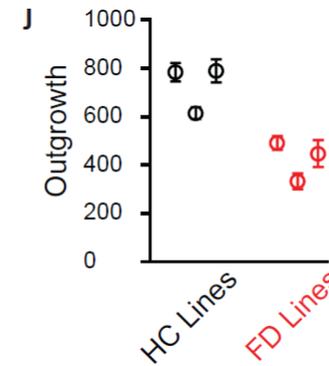
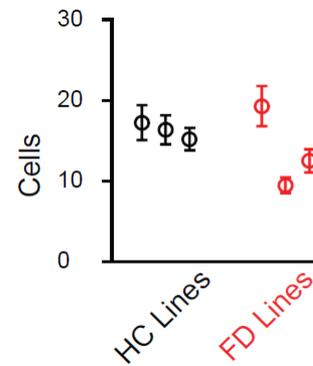
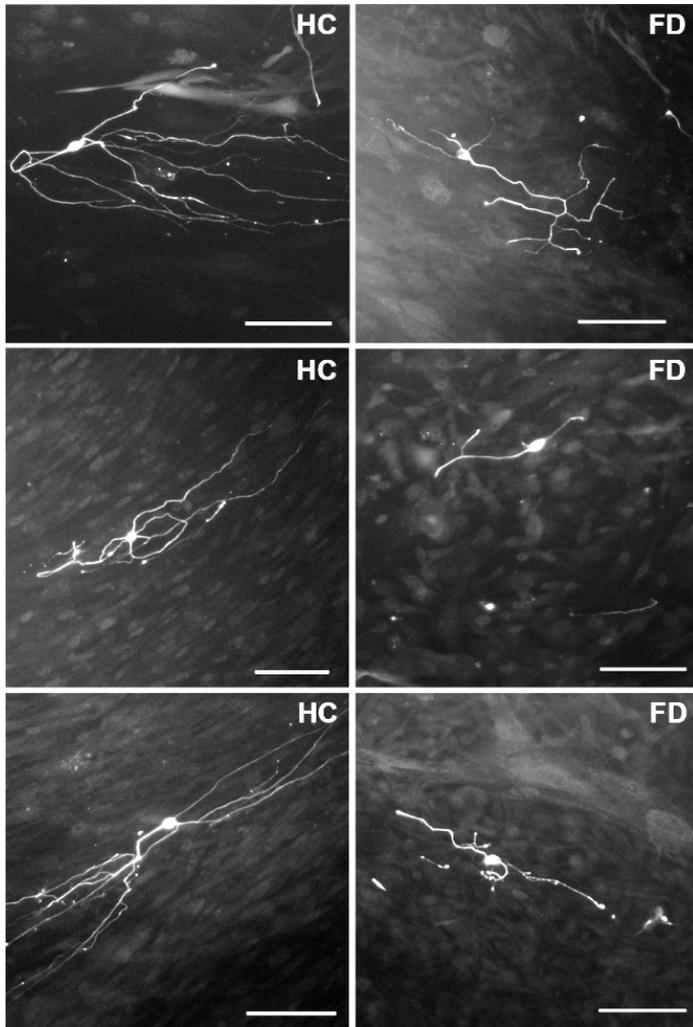
0.1



Induced Human neurons

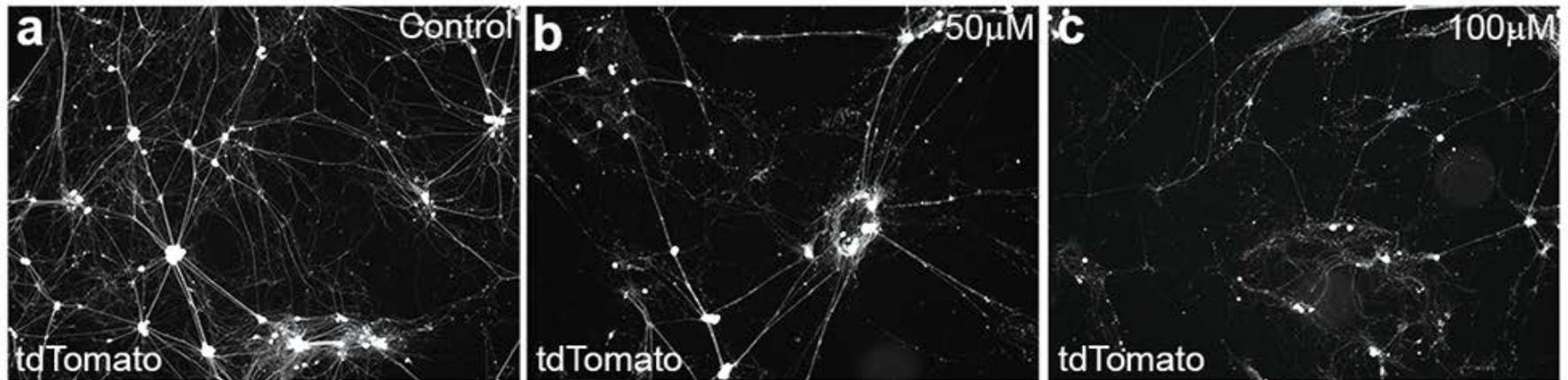


Induced neurons from a patient with FD differ from healthy control induced neurons



Induced neurons can be used to screen for neurotoxic effects of cancer chemotherapeutic agents

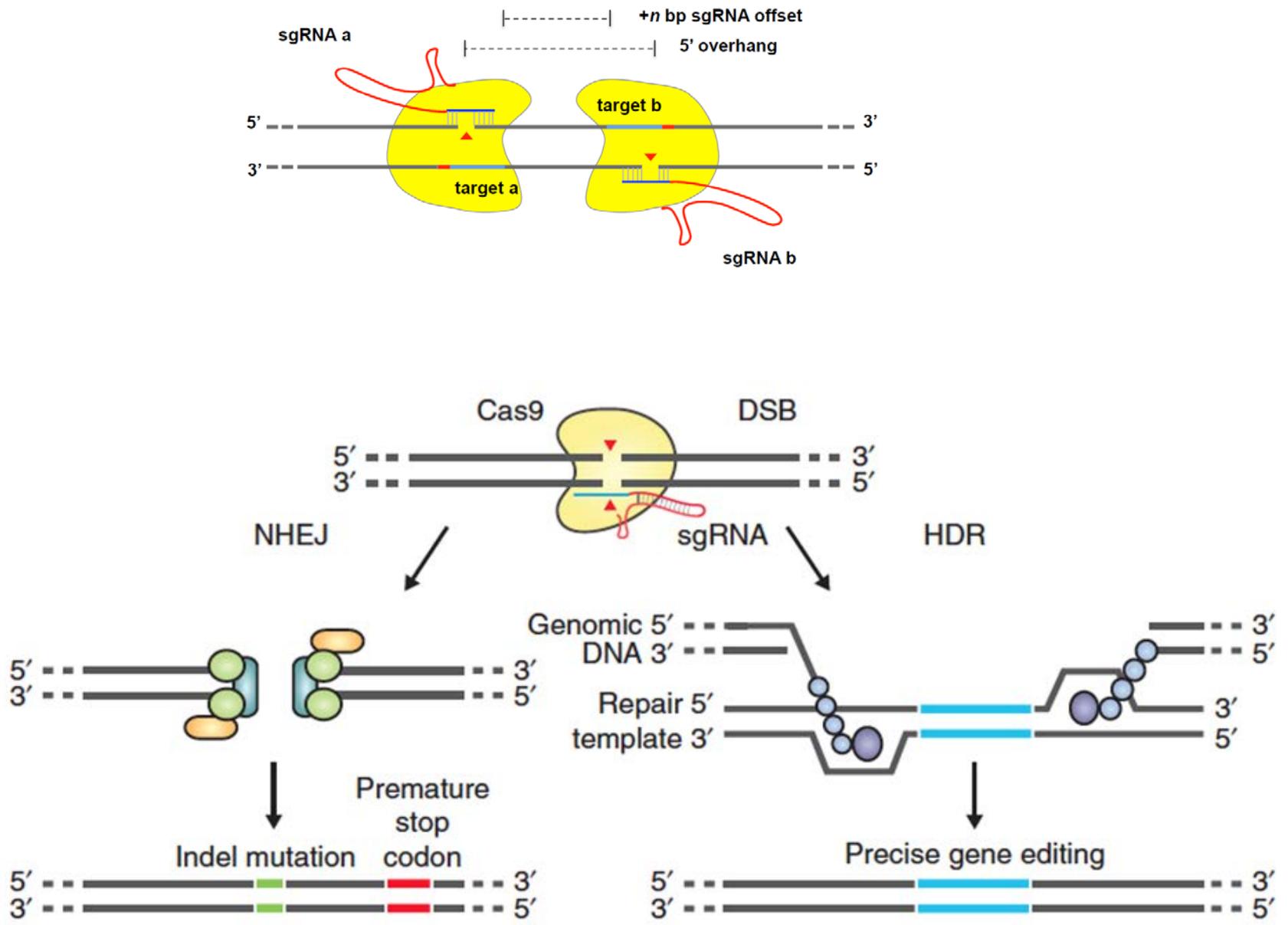
oxaliplatin



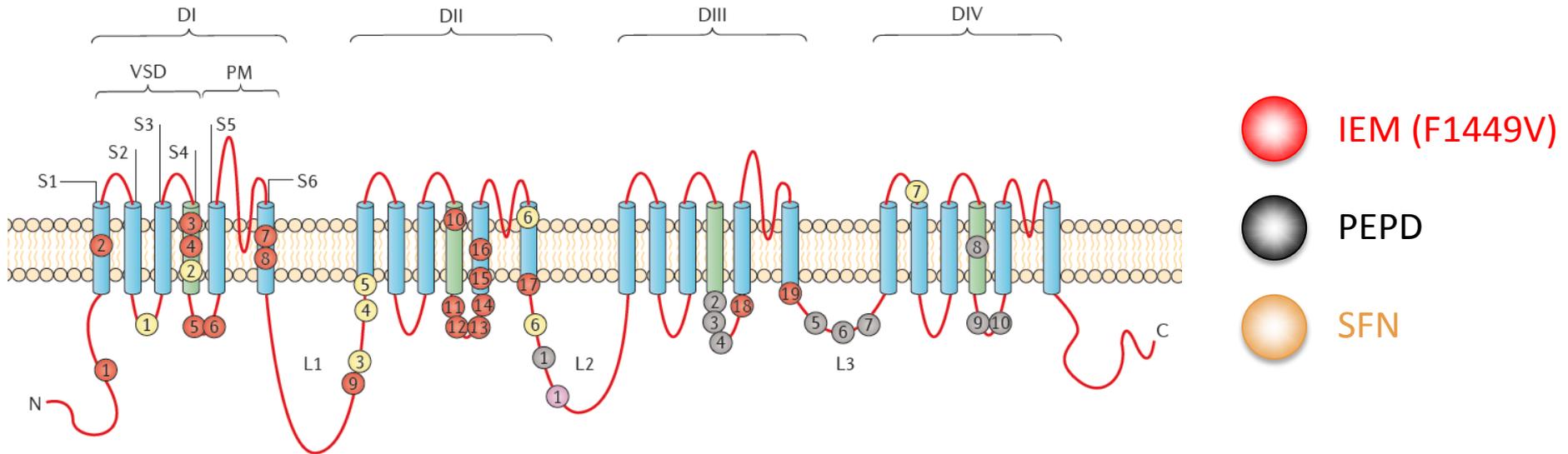
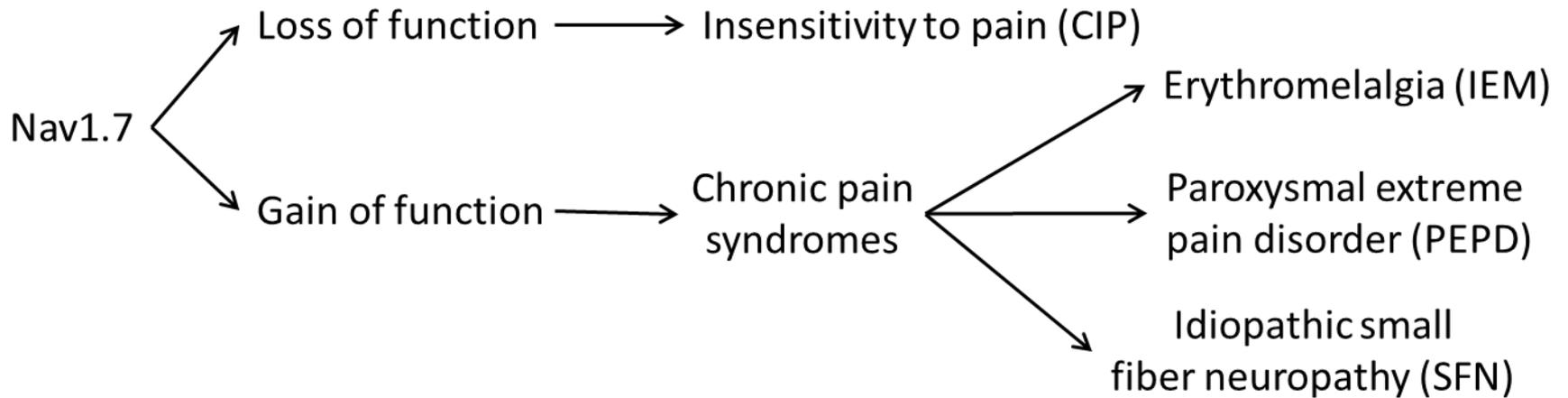
Modeling pain *in vitro* using nociceptor neurons reprogrammed from fibroblasts

Brian J Wainger^{1-3,8}, Elizabeth D Buttermore^{1,3,8}, Julia T Oliveira^{1,4}, Cassidy Mellin¹, Seungkyu Lee^{1,3}, Wardiya Afshar Saber¹, Amy J Wang¹, Justin K Ichida^{5,6}, Isaac M Chiu^{1,3}, Lee Barrett¹, Eric A Huebner^{1,3}, Canan Bilgin¹, Naomi Tsujimoto⁵, Christian Brenneis¹, Kush Kapur¹, Lee L Rubin⁵, Kevin Eggan^{5,7} & Clifford J Woolf^{1,3}

A



Nav1.7 mutations





Brian Wainger



Liz Buttermore



Julia Oliveras



Cassidy Mellin

Justin Ichida
Wardiya Afshar
Isaac Chiu

Lee Rubin
Kevin Eggan



Cedric Laedermann
Inge Chen
Lendy Chu