Microglial regulation of hyperexcitability in cortical neurons derived from human iPSCs carrying epilepsy-associated sodium channel Nav1.2 variant

Zhefu Que[1], **Maria Olivero-Acosta**[1], Jingliang Zhang[1], Kyle W Wettschurack[1], William Skarnes[2], Yang Yang[1]*;
Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University[1]. The Jackson Lab[2]

Advancing pharmacogenomics to cure diseases of the nervous system and cancer

Introduction

Nav1.2, a voltage-gated sodium channel encoded by *SCN2A*, is responsible for action potential firing and propagation in the central nervous system. Due to the wide adoption of whole-genome sequencing over the past few years, a strong correlation has been established between genetic variants of *SCN2A* and a wide range of neurological diseases including epilepsy, autism spectrum disorder (ASD) and intellectual disability among others *(Begemann et al., 2019)*. To understand how major recurring variants of *SCN2A* perturb neurons and alter neuronal excitability, we used CRIPSR/Cas9 to create an *SCN2A* disease-associated variant: p.L1342P in a human reference iPSC line (KOLF2-C1), a line with defined genetic lineage, reducing unwanted influence by patient-specific backgrounds. This recurring variant detected in 5 patients *(Matalon, 2014; Hackenberg, 2014; Dimassi, 2016; Wolff, 2017)*, has been associated with early-onset epileptic encephalopathy, which manifests as very severe and intractable seizures. The data we present will validate the feasibility of using iPSC disease models to elucidate mechanisms of disease specifically caused by *SCN2A* variants and provide information for personalized treatment (for detailed information, please refer the paper recently published on *Journal of Neuroscience*).

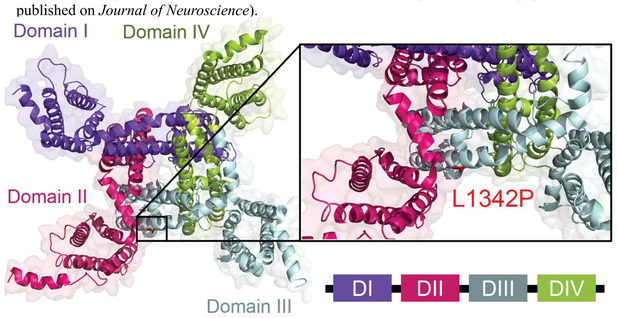


Figure 1. Schematic 3D-Structure of Nav1.2 Sodium Channel. Mutated residue L1342P is indicated by zoomed-in box. Modified from PDB ID: 6J8E *(Pan et al., 2019)*

Methods and results

Significance statement: A mounting number of SCN2A genetic variants have been identified from patients with epilepsy, but how SCN2A variants affect the function of human neurons contributing to seizures is still elusive. This study investigated the functional consequences of a recurring SCN2A variant (L1342P) using human iPSC-derived neurons and revealed both intrinsic and network hyperexcitability of neurons carrying a mutant Nav1.2 channel. Importantly, this study recapitulated elements of clinical observations of drug-resistant features of the L1342P variant, and provided a platform for in vitro drug testing. Our study sheds light on cellular mechanism of seizures resulting from a recurring Nav1.2 variant, and helps to advance personalized drug discovery to treat patients carrying pathogenic SCN2A variant.

Schematic of a modified DUAL-SMADi differentiation protocol and the timeline for experiments

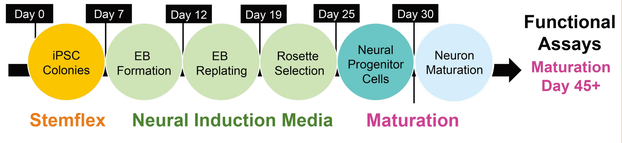
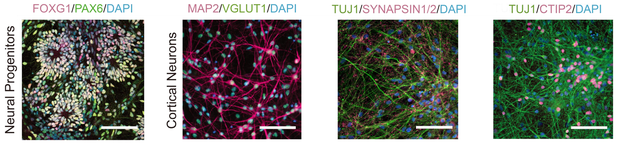


Figure 2. Flowchart depicting the stepwise differentiation protocol. Reference line KOLF2-C1 iPSCs were differentiated for 45 days into cortical neurons.

iPSC-derived neurons express cortical neuron specific markers



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Results

Nav1.2-L1342P variant displays complex gating kinetics

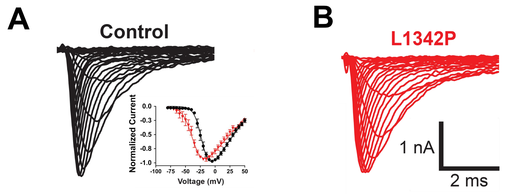


Figure 4. Representative traces of whole-cell sodium current from HEK cells expressing control Nav1.2 (A) or Nav1.2-L1342P channel (B). The inset depicts the normalized current-voltage (I-V) curve.

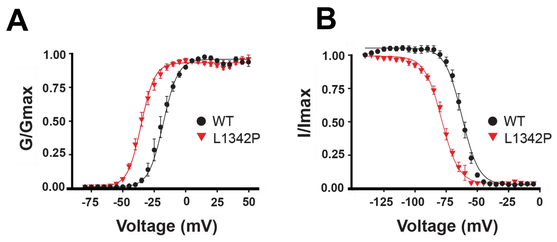


Figure 5. Voltage-dependent activation (A) and steady-state fast inactivation (B) of WT and L1342P-HET mutant channels. (A) L1342P variant causes a left-shift around 17 mV, indicating a gain-of-function phenotype. **(B)** In fast-inactivation, a left-shift indicates a loss-of-function phenotype.

The L1342P variant increases the intrinsic excitability of hiPSC-derived neurons

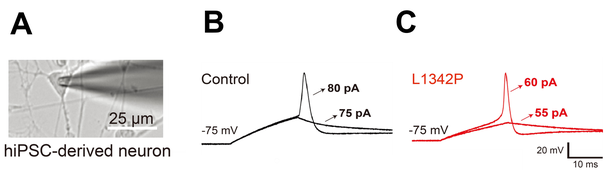
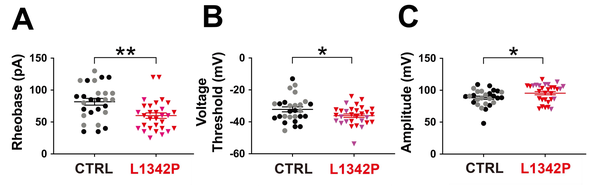


Figure 6. Representative action potential firings from iPSC-derived neurons. (A) Representative brightfield image of a hiPSC-derived pyramidal-shaped neuron selected for patch-clamp experiments. **(B)** and **(C)** Representative single action potential triggered by a stepwise increment of current stimulus with a 20 ms duration.



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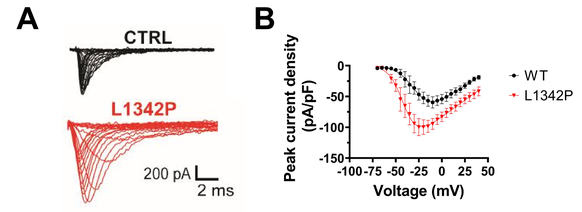

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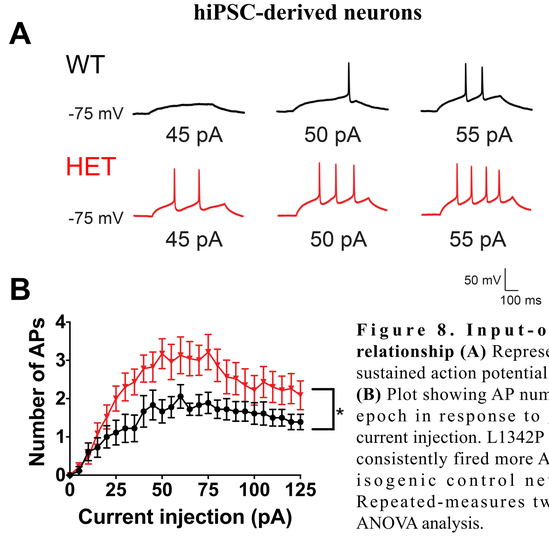

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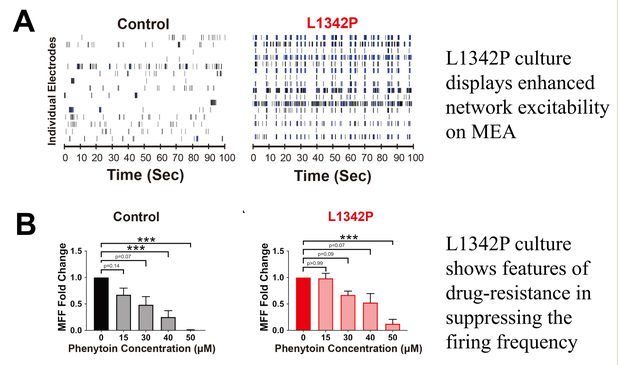
Heightened neural network excitability, with reduced sensitivity in phenytoin treatment using MEA in hiPSC-derived neurons carrying the Nav1.2-L1342P variant



L1342P culture displays enhanced network excitability on MEA

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Characterization of hiPSC-derived microglia and co-culture with iPSC-derived neurons

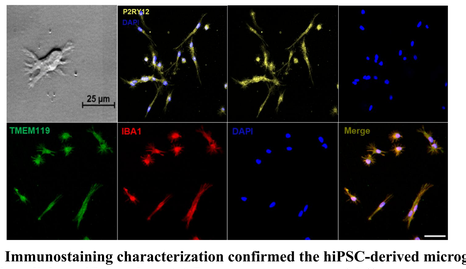

Figure 10. Immunostaining characterization confirmed the hiPSC-derived microglia express microglial specific markers (A) Representative images of differentiated microglia from hiPSC expressing microglial markers: P2RY12 (yellow), TMEM119 (green), IBA1 (red). Scale bar = 50 µm.

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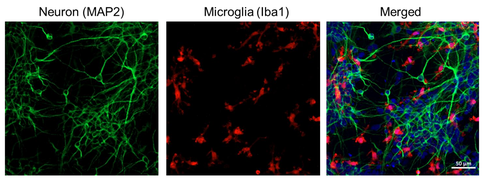

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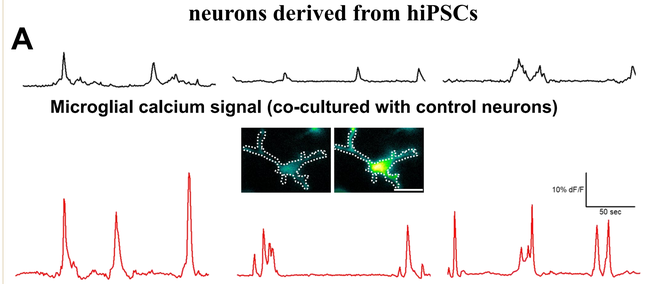
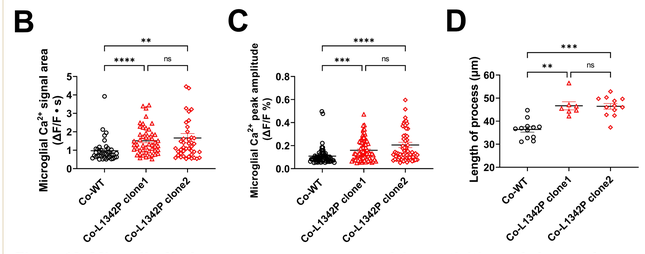
Enhanced calcium signal and more extended process of microglia co-cultured with endogenously hyperexcitable L1342P neurons derived from hiPSCs





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Conclusions

- ❑ L1342P variant displays complex biophysical property in voltage-clamp mode.
- ❑ Neurons with L1342P variant displays increased intrinsic and network excitability, with elevated current density, indicating a gain-of function phenotype.
- ❑ hiPSC-derived microglia cocultured with L1342P neuron culture shows increased calcium activities and elongated process.

References

- Dimassi, S., A. Labalme, D. Ville, A. Calender, C. Mignot, N. Boutry- Kryza, J. De Bellescize et al. "Whole- exome sequencing improves the diagnosis yield in sporadic infantile spasm syndrome." Clinical genetics 89, no. 2 (2016): 198-204.
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Acknowledgements

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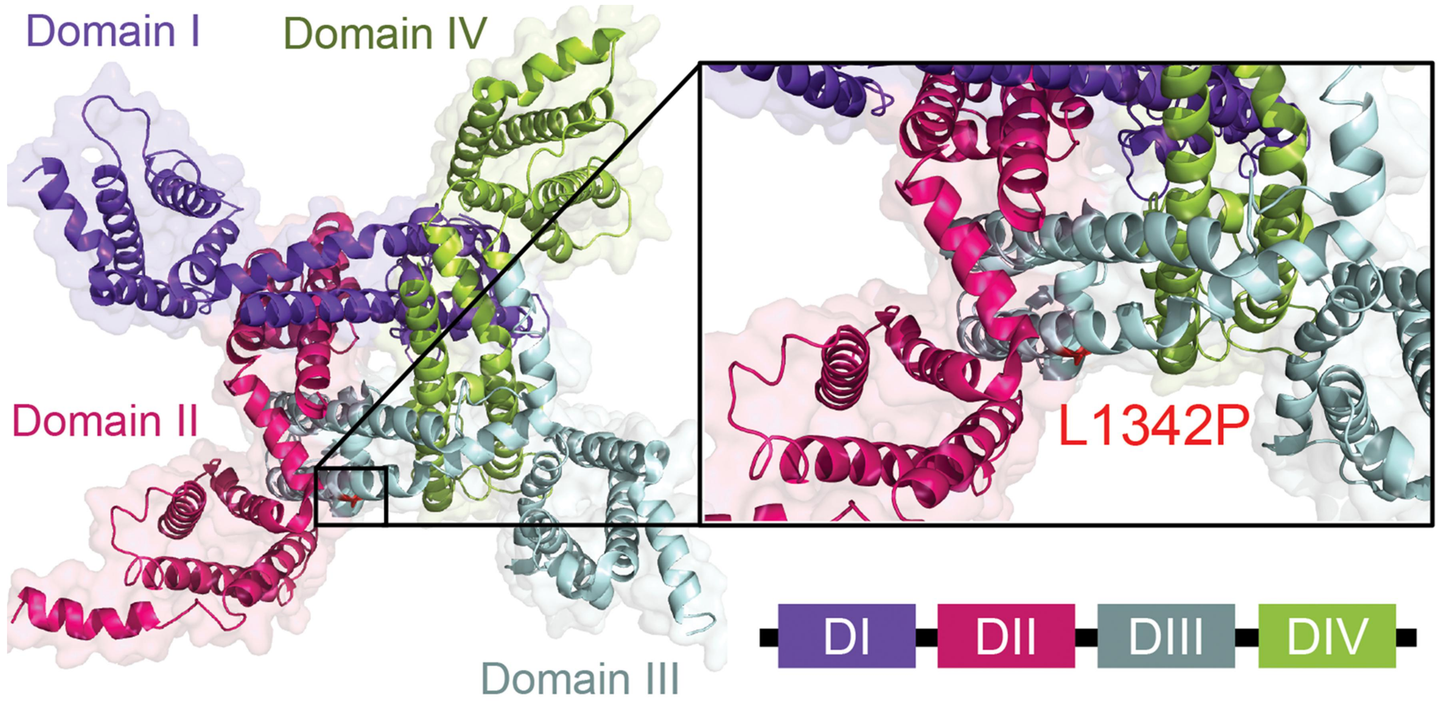

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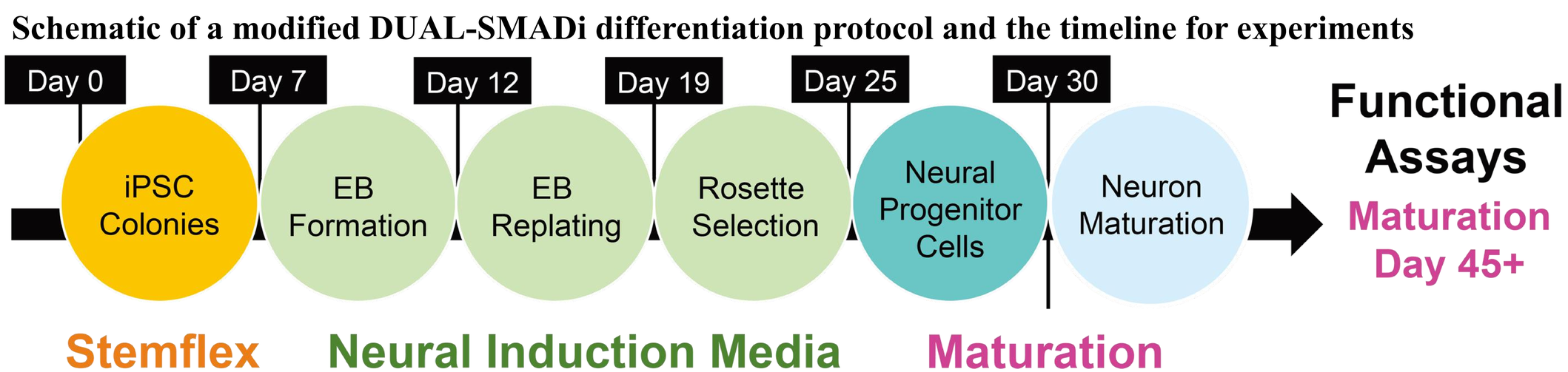
Timeline and characterization of hiPSC-neurons



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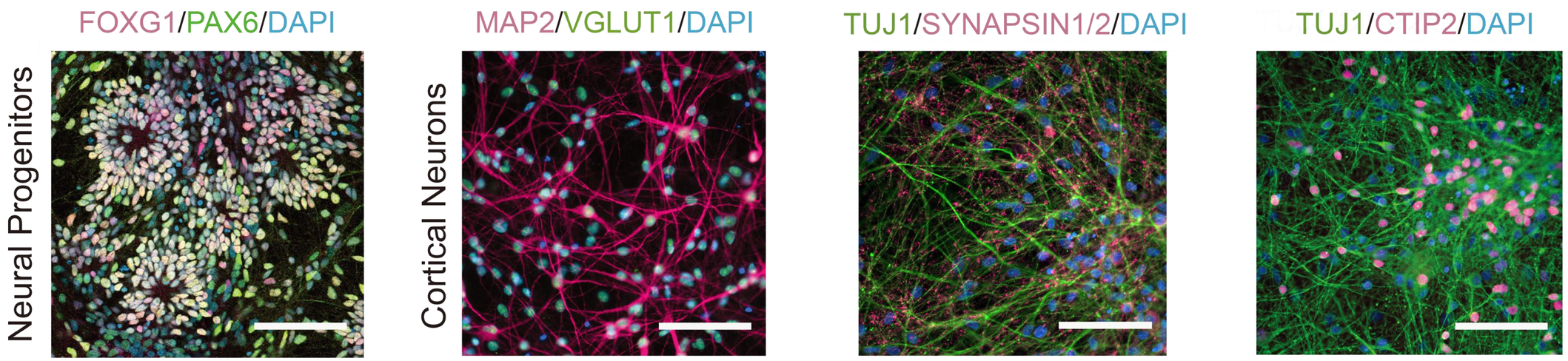

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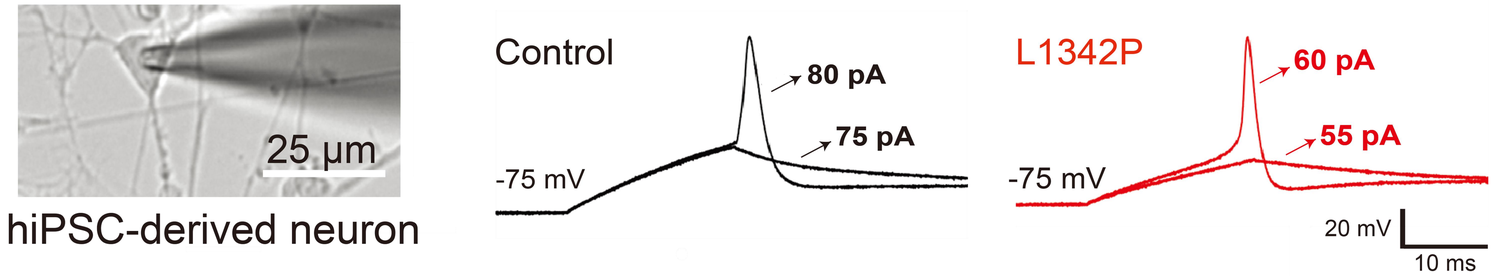

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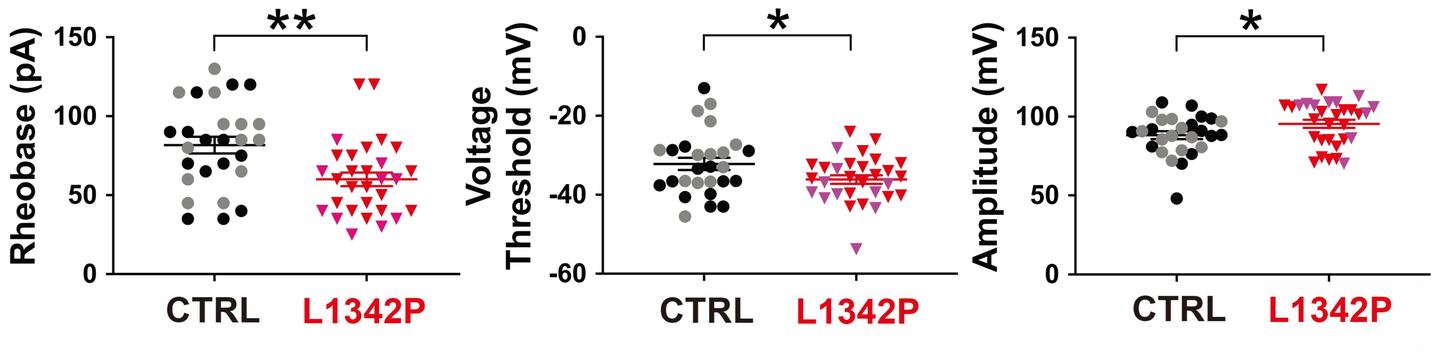

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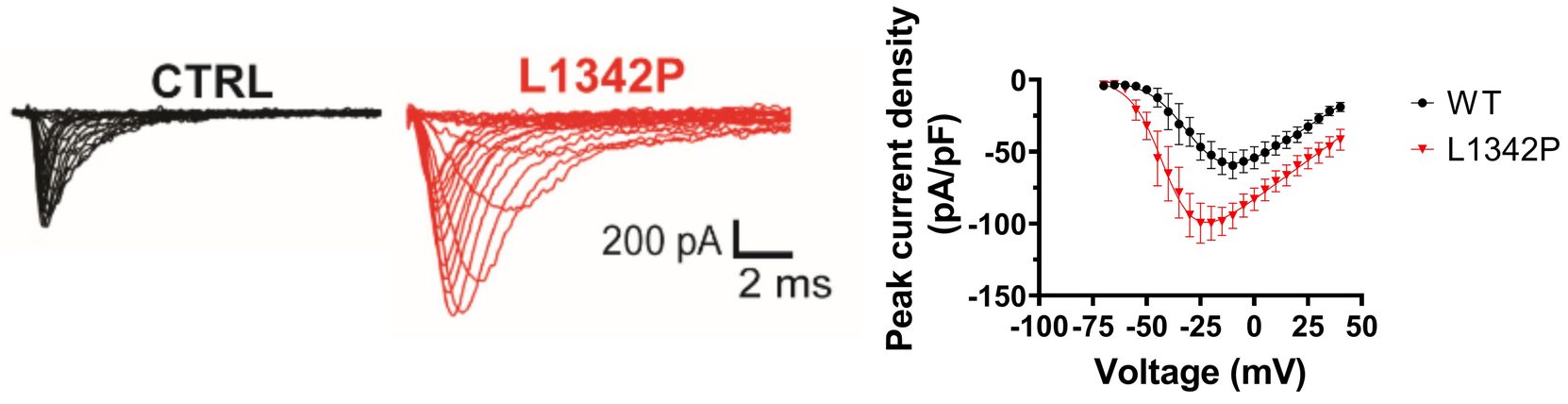

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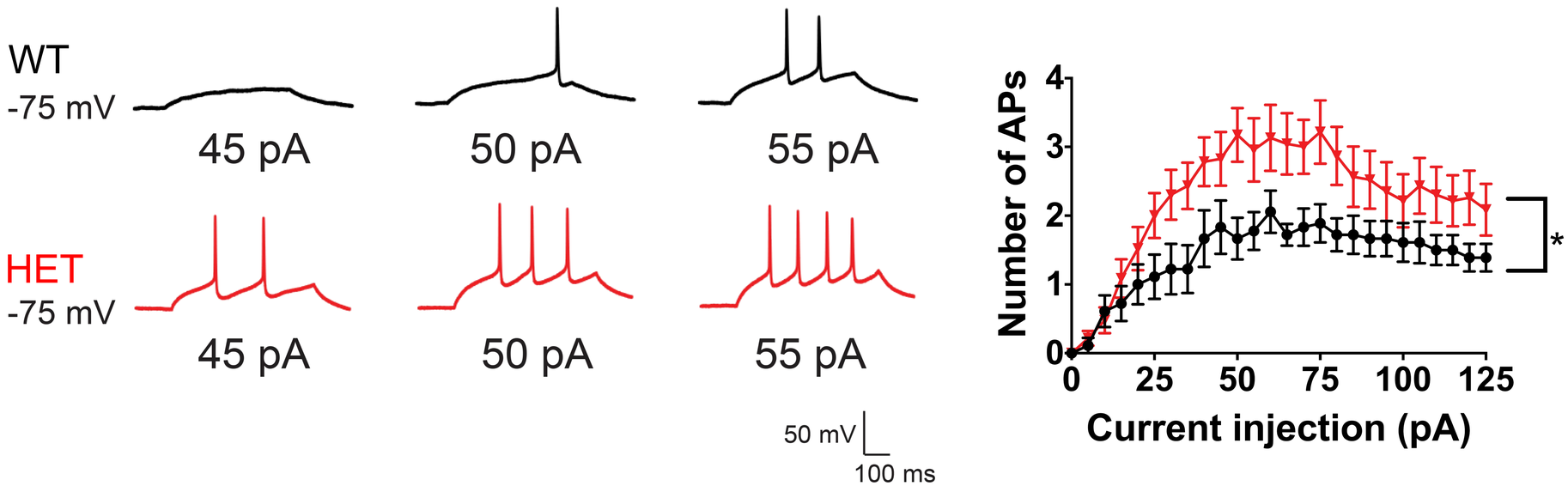

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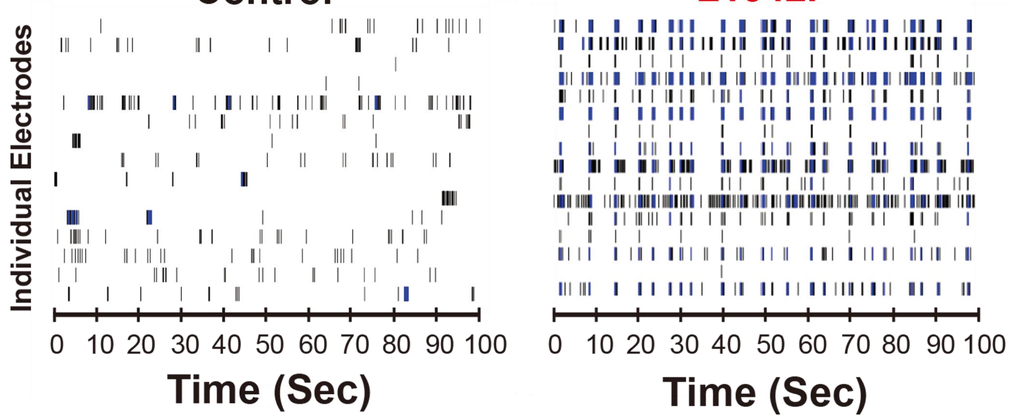

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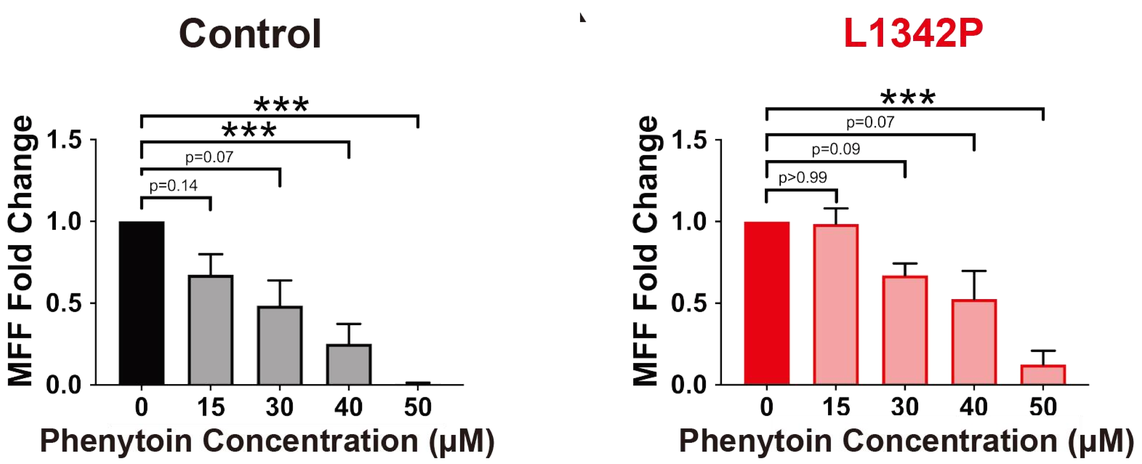

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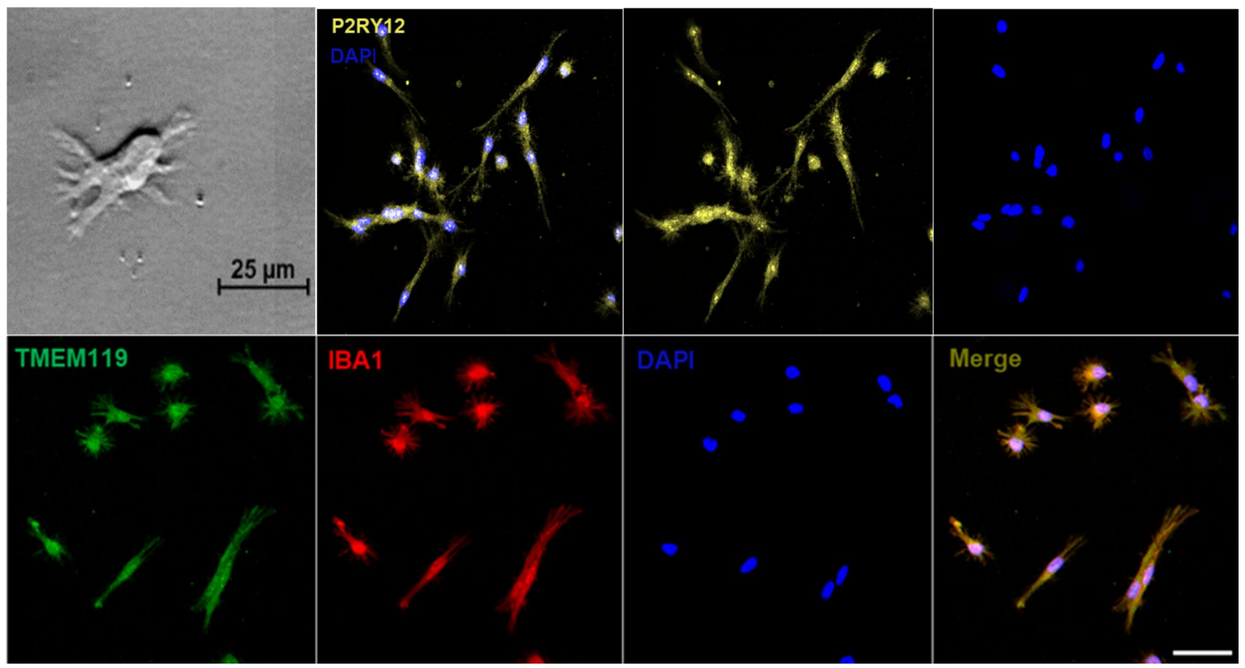

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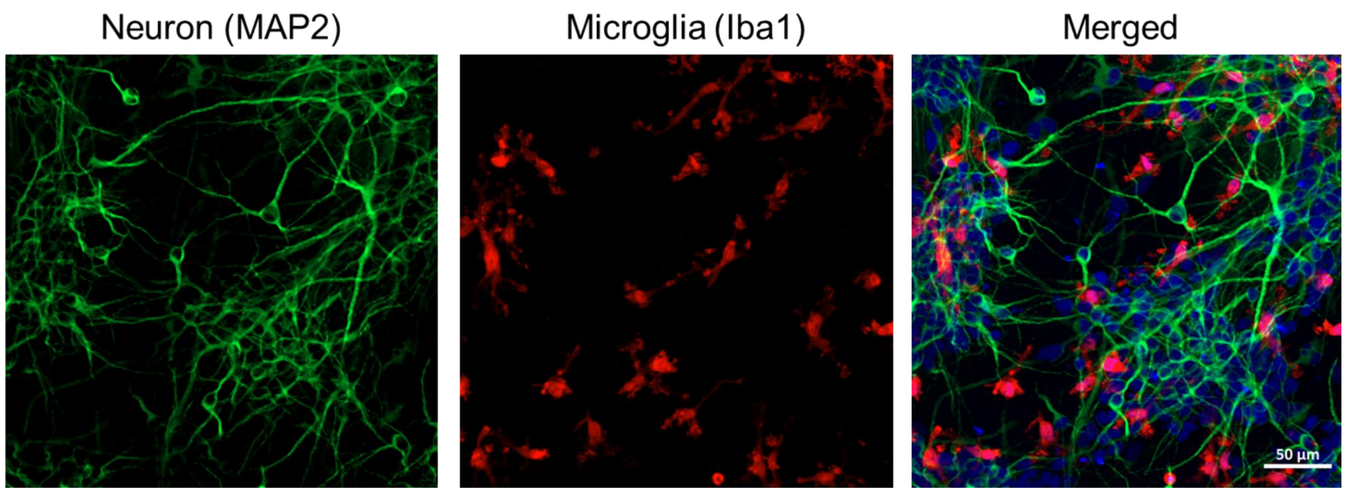

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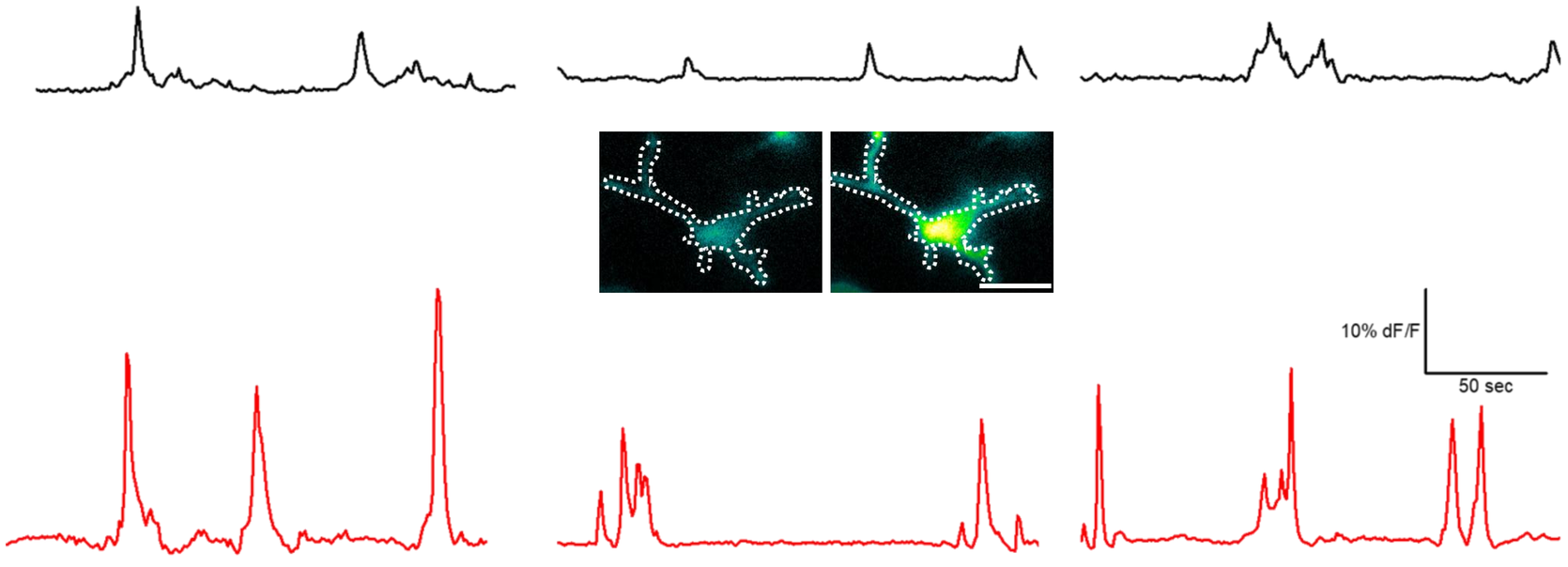

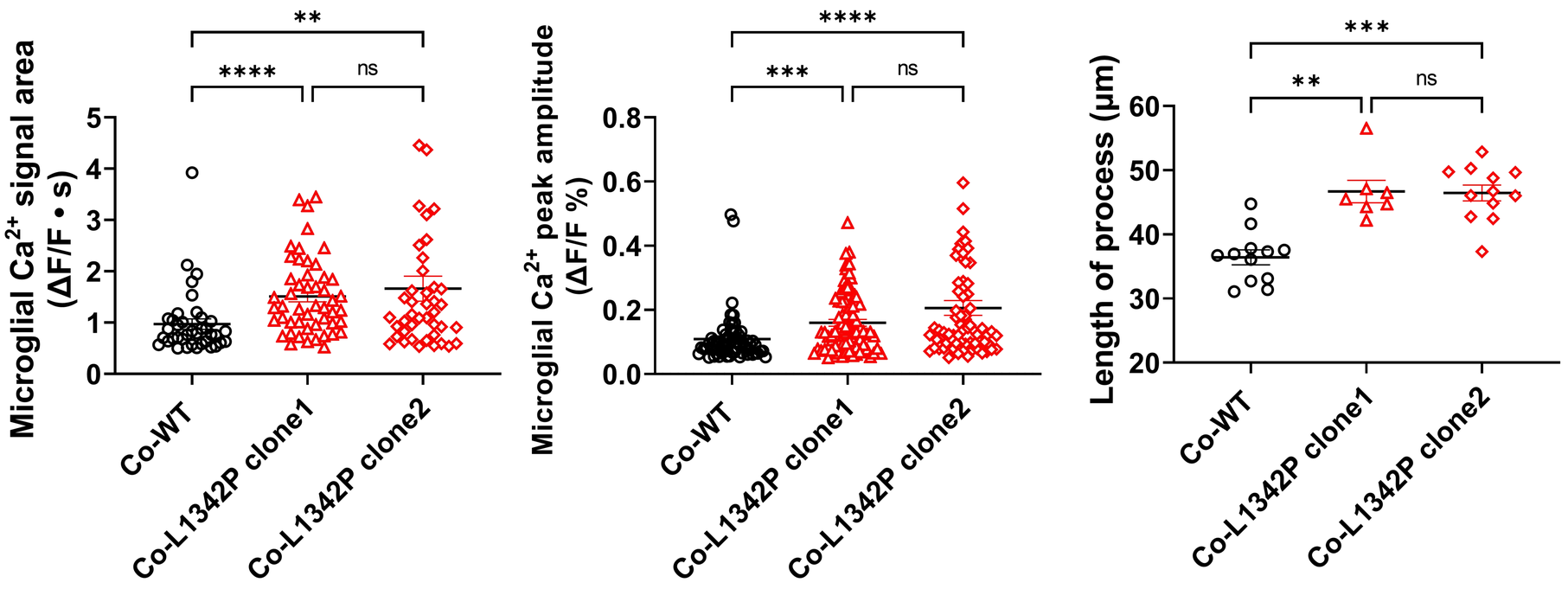

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