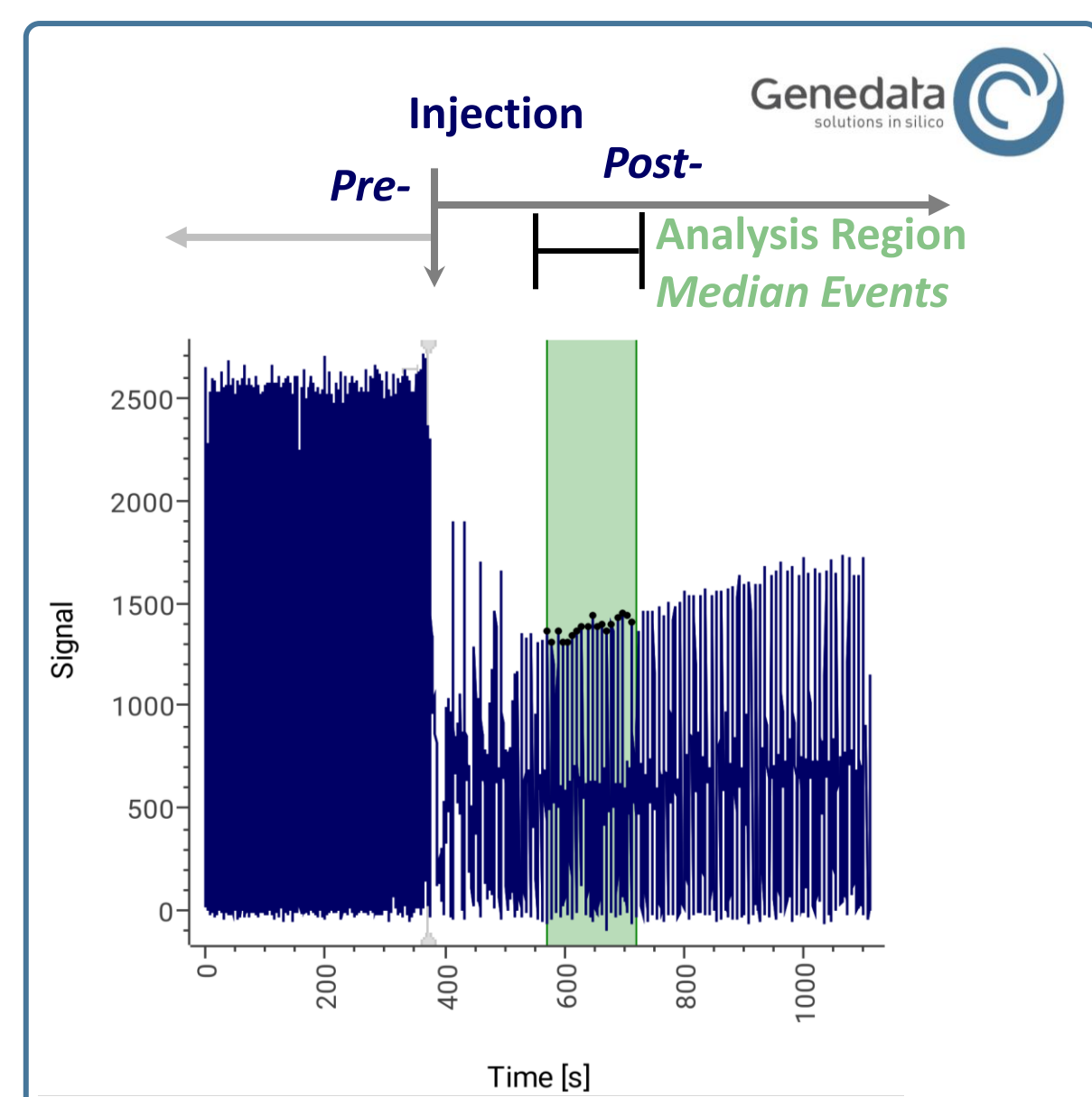
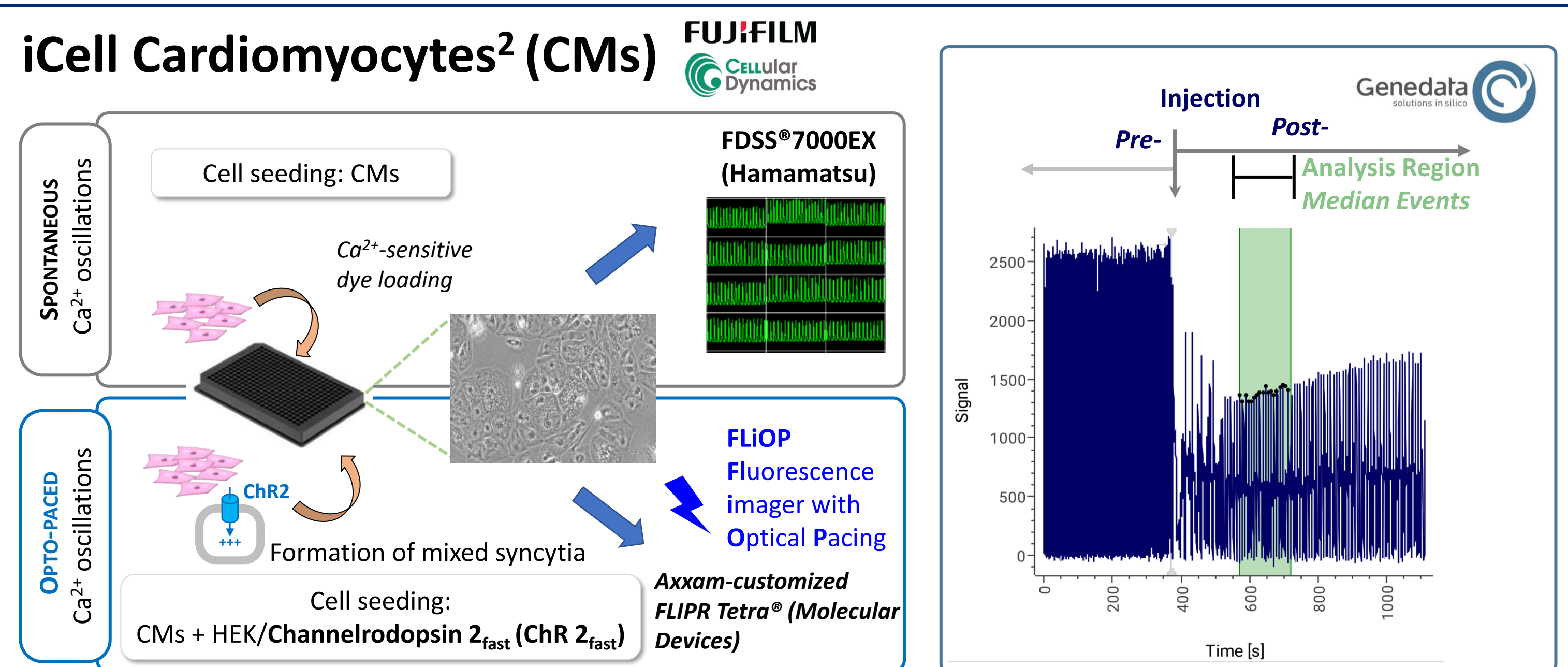


# Riding the Calcium Wave: High-Throughput Insights from Spontaneous and Induced Ca<sup>2+</sup> Oscillations using Relevant iPSC-based Models

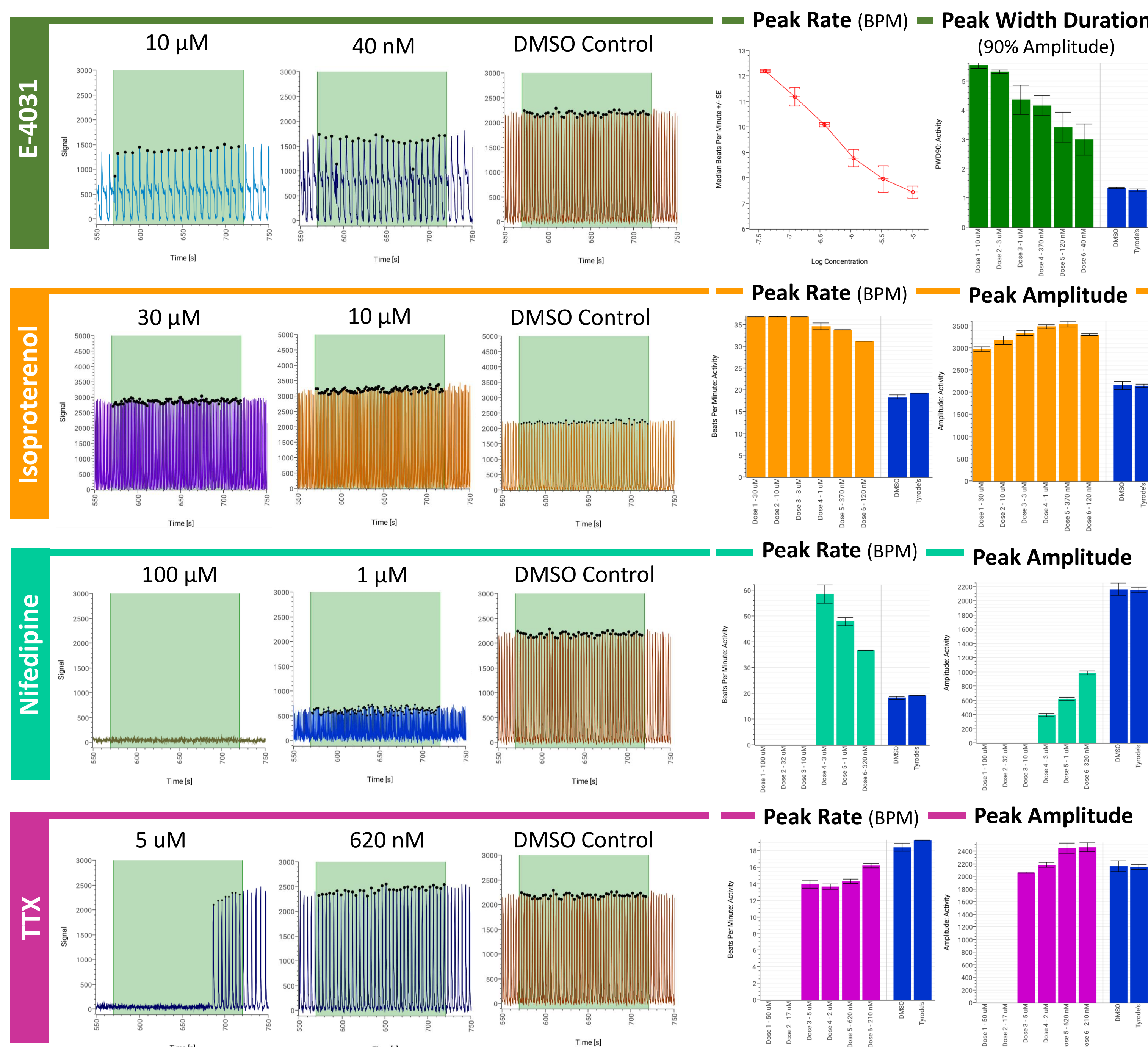
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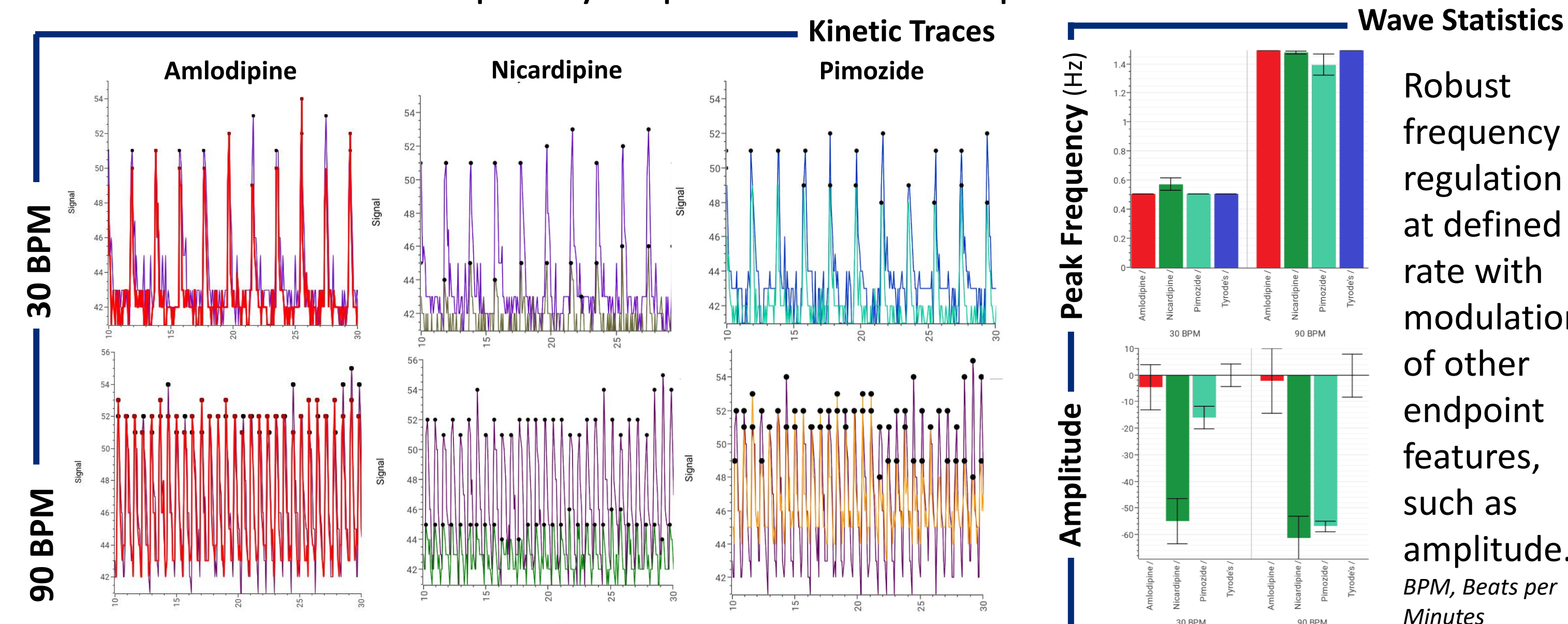
Calcium (Ca<sup>2+</sup>) oscillations are ubiquitous biological signals that are important for many intracellular functions and may vary in different physiological or pathological conditions. Accumulation of Ca<sup>2+</sup> in cardiac cells is the trigger for contraction and modulates several transmembrane currents; dysregulations in Ca<sup>2+</sup> handling have been associated with the appearance of arrhythmias. Moreover, unwanted drug interactions with cardiac ion channels can lead to increased pro-arrhythmic risk and it is a priority to detect these interactions as early as possible during drug development. Spontaneous Ca<sup>2+</sup> oscillations usually occur in neural networks and are closely tied to neuronal activity, with alterations in Ca<sup>2+</sup> homeostasis having been observed in neurodegenerative pathologies. Herein, we describe the development of HTS-compatible Ca<sup>2+</sup> oscillation assays at FDSS®7000EX (Hamamatsu), FLIPR Tetra® (Molecular Devices) and FLiOP using hiPSC-derived neurons and cardiomyocytes (Fujifilm CDI). We have investigated the application of hiPSC platforms for disease modelling to be deployed for screening compound-induced effects and enable drug discovery against neurological disorders. We demonstrate the use of a new analysis workflow available through Genedata Screener® that allows automated high-throughput data analysis and reduces a common bottleneck in the adoption of hiPSCs and Ca<sup>2+</sup> oscillation assays.



**SPONTANEOUS Ca<sup>2+</sup> OSCILLATION ASSAY:** E-4031, Isoproterenol, Nifedipine and TTX were tested in dose-response. Ca<sup>2+</sup> oscillations recorded at FDSS®7000EX are consistent and reproducible and can be modulated with compound treatments.

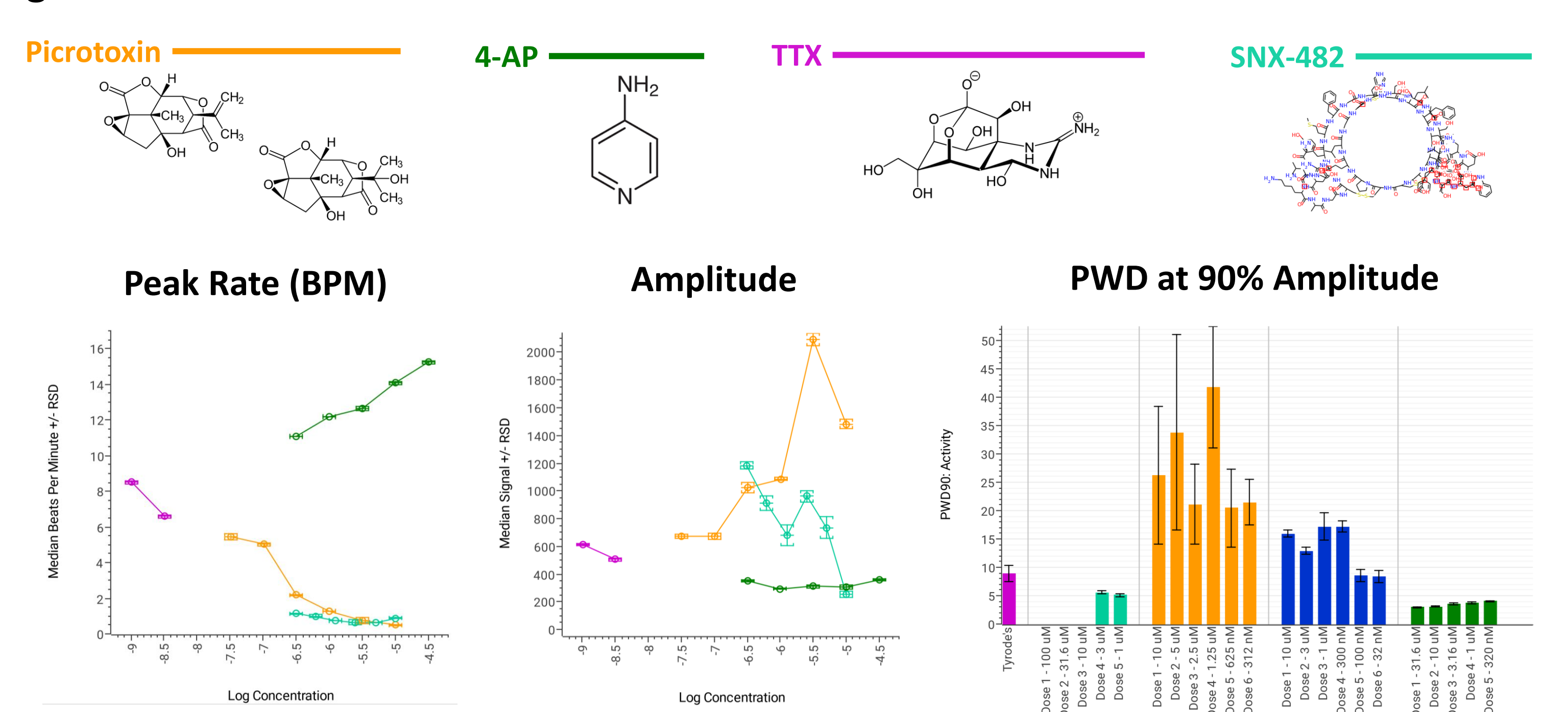


**OPTO-PACED Ca<sup>2+</sup> OSCILLATION ASSAY:** CMs and HEK-293 cells expressing ChR2<sub>fast</sub> variant were co-seeded to form syncytia. Light pulse stimulation of syncytia at FLiOP lead to synchronized and controlled Ca<sup>2+</sup> oscillations. Ca<sup>2+</sup> channel blockers tested showed both frequency-dependent and independent effects.

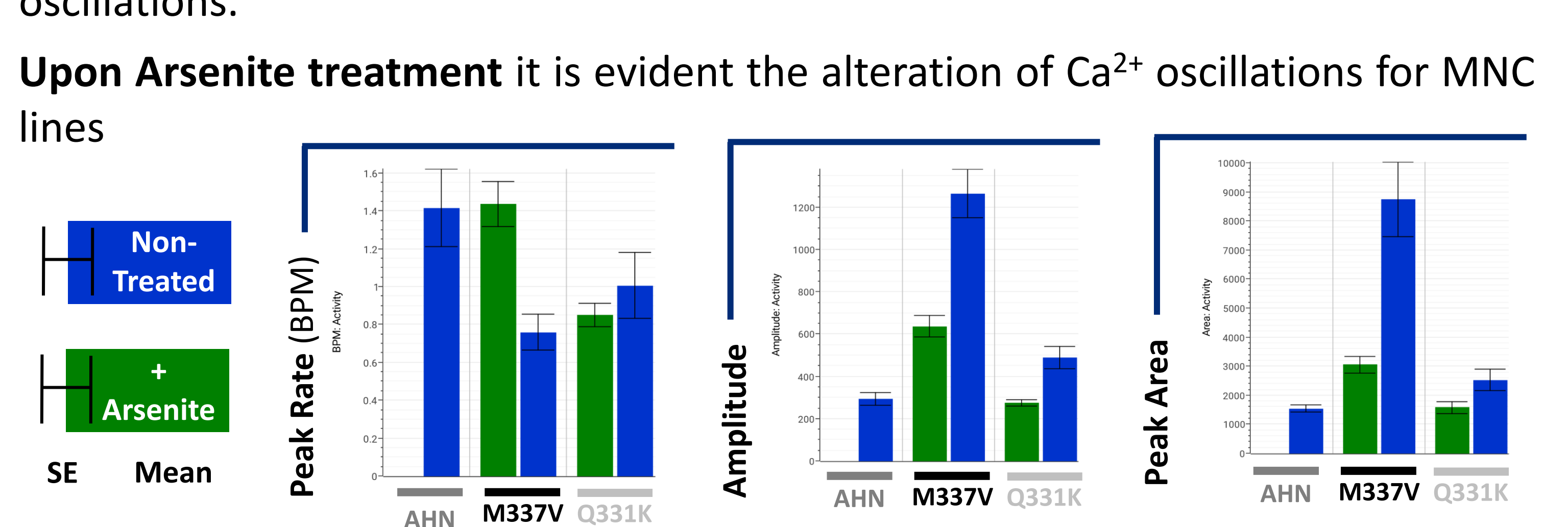


Robust frequency regulation at defined rate with modulation of other endpoint features, such as amplitude. BPM, Beats per Minutes

**iCell GlutaNeurons (GNCs)** display spontaneous Ca<sup>2+</sup> oscillations that can be exploited for toxicology testing and disease modelling. Potentially seizurogenic compounds could modulate network activity of GNCs concentration-dependently. Several oscillation parameters, including frequency and amplitude, can be easily analyzed with Genedata Screener® and visualized dynamically for insight generation.



**iCell MotorNeurons (MNCs)** Different patterns of Ca<sup>2+</sup> oscillations are observed in apparently healthy normal (AHN) and TDP43-mutant MNCs, showing mutation-specific phenotypes, suitable for disease modeling. A persistent and mild oxidative stress insult by Arsenite perturbs Ca<sup>2+</sup> oscillations.



Upon Arsenite treatment it is evident the alteration of Ca<sup>2+</sup> oscillations for MNC lines

These data represent a comprehensive *in vitro* platform for automated drug screening purposes which allows the measurement and analysis of Ca<sup>2+</sup> oscillations. The optimized assays allow both profiling for safety/toxicology studies and modelling disease phenotypes. We have demonstrated:

- Use of homogenous and reproducible hiPSC-derived neuron and cardiomyocyte models (FCDI)
- Readiness for automation with optimized culture and assay conditions in 384-well plate format
- Efficient high-throughput data analysis validated in Genedata Screener® allowing rapid and robust data turnaround in both standardized and exploratory projects