Using hiPSC-Derived Neuronal Cultures To Assess The Safety Of Pre-Screened Potential Therapeutic Compounds Against Zika Virus

Ileana Slavin[1], Steven Dea[1], Sandro Montefusco[2], Jair Siqueira-Neto[2], Fabian Zanella[1] and Cassiano Carromeu[1]

1 - StemoniX, San Diego, CA; 2 - University of California, San Diego (UCSD)
www.stemonix.com - Contact info: info@stemonix.com

Abstract
We present a human cell-based screening platform to investigate the toxicological profile of potential therapeutic compounds affecting the Central Nervous System. By using a hiPSC-derived neural model, we tested the safety profile of 29 compounds recently described as potential drug treatments to Zika Virus infection. Our screening system was able to detect strong toxicity for some drugs during early stages of human nervous system development, with decreased toxicity as the cells matured *in vitro*. The present work highlights the power of a human neural screening system and its use in assessing the toxicological profile of potential therapeutic compounds.

Introduction
The recent global threat of the Zika Virus epidemic has highlighted the need for sophisticated screening systems capable of detecting unintended toxicity of candidate therapeutic compounds. Toxicity to the Central Nervous System (CNS) is a key step in the safety pharmacology evaluation of drugs under development. The characterization of toxicological profiles of chemical compounds in the CNS involves extensive investigation using *in vitro* and *in vivo* models. Human induced pluripotent stem cell (hiPSC) technology has enabled the readily availability of large and consistent batches of neural cells for wider toxicity screenings. These valuable cellular models hold great potential in reducing not only the cost but also the time to assess toxicity of developing drugs. By using a hiPSC-derived neural model, we investigated the toxicological profile of 29 compounds recently described in the literature as potential therapeutic compounds against Zika Virus infection.

Methods
Commercially available StemoniX Neural Progenitor Cell (NPC) lines were used to generate populations of cells representing different neural developmental stages: from neural progenitor cells to mature neural cultures (up to 8 weeks old *in vitro*). Cell populations were then subjected to two different cell viability assays, Resazurin (PrestoBlue, Thermo) and ATP (CellTiter-Glo, Promega), at different developmental times (neural progenitors and 2, 4 and 8 weeks of differentiation). We also investigated the toxicity in 3D organoid cultures (microBrain). Candidates with the safest cell viability profiles were further evaluated using high throughput calcium flux (FLIPR[Tetra] from Molecular Devices) and multi-electrode array (Maestro from Axion Biosystems) assays for assessment of potential functional side effects on the CNS.

Results

StemoniX NPCs

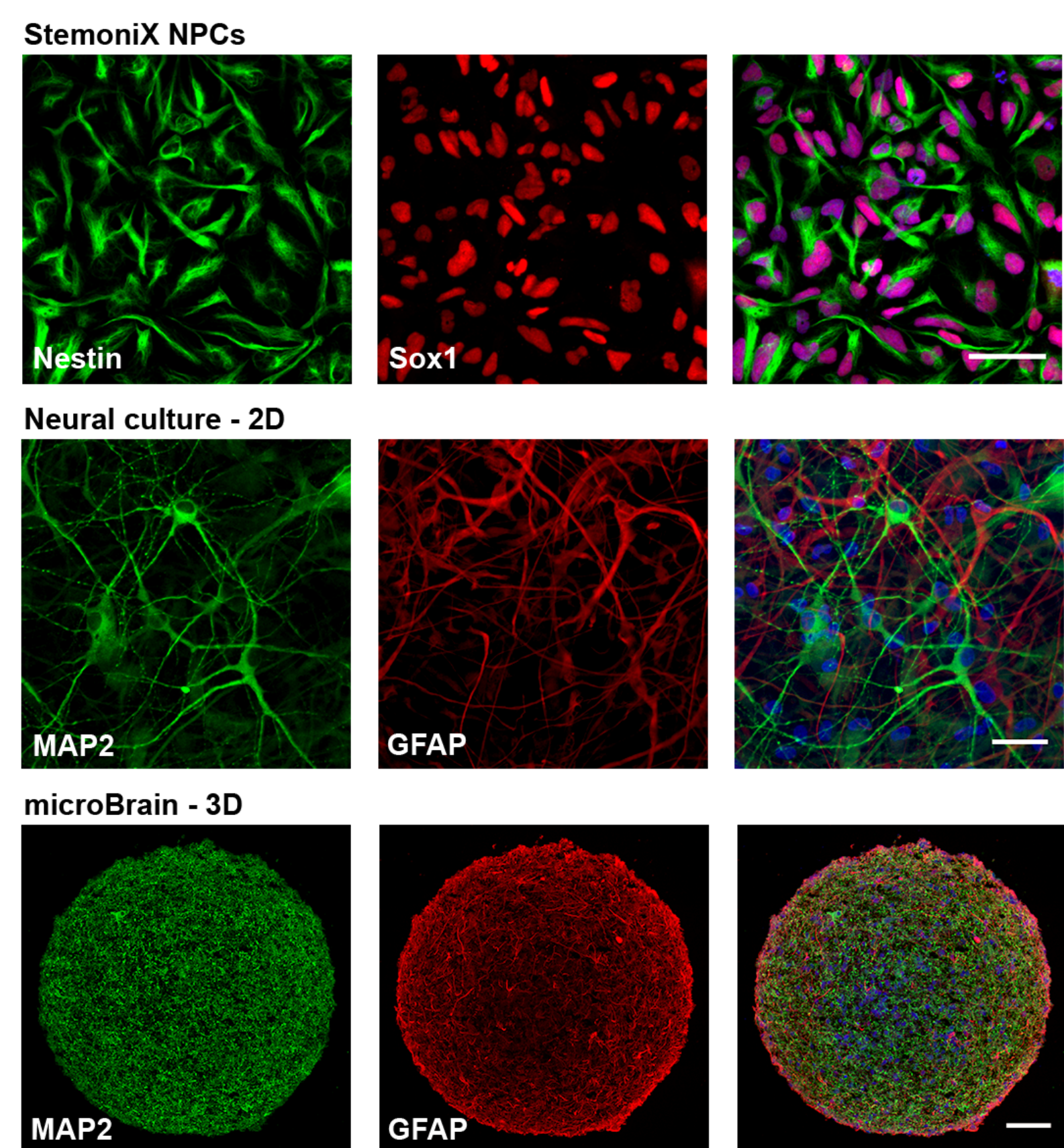### Neural culture - 2D

microBrain - 3D

Figure 1: Immunostaining of neural cultures at different stages of development. Scale bar for NPCs and Neural culture 2D are 50µm. Scale bar for microBrain is 100µm.

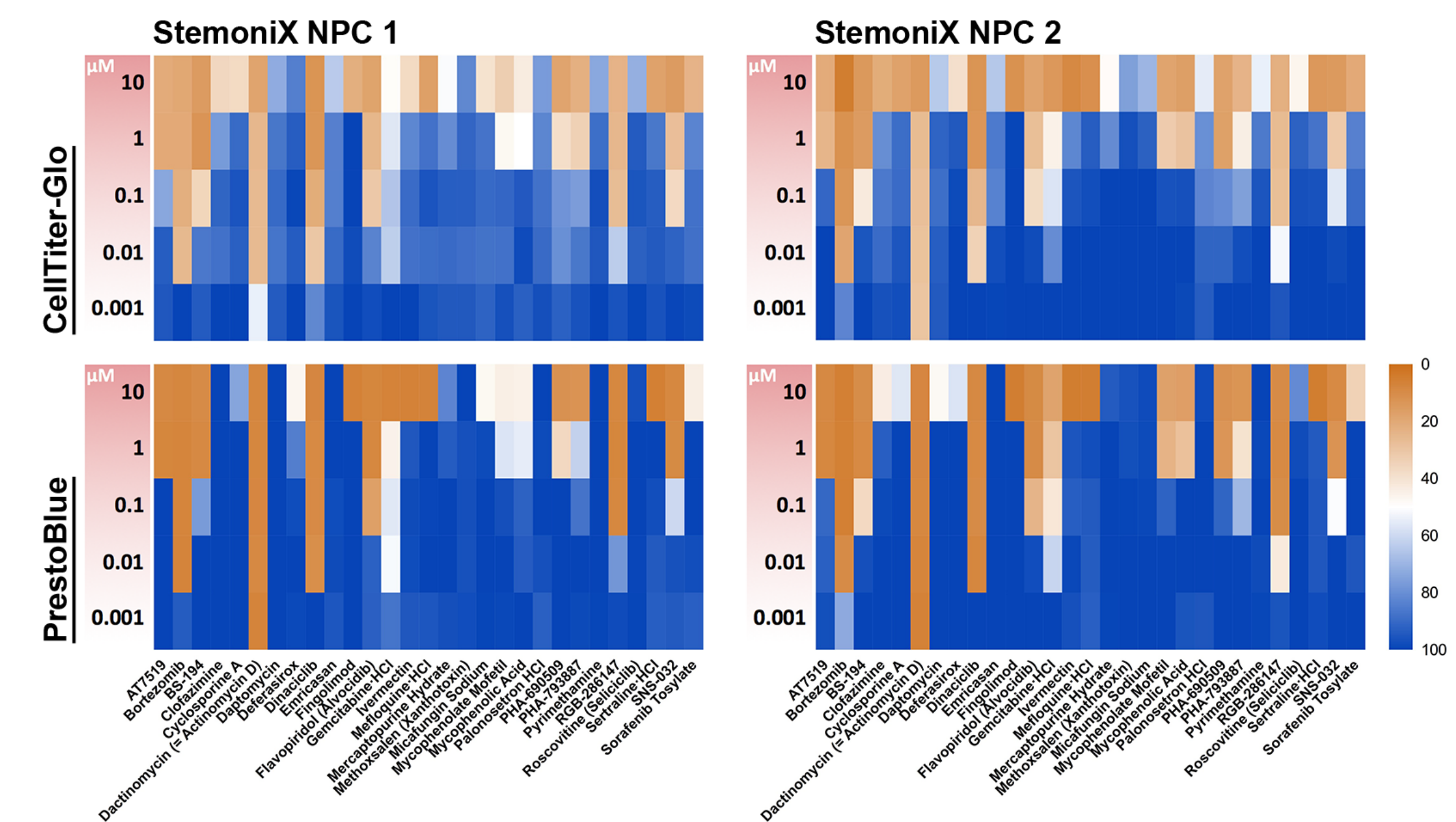### Cell Viability of Neural Progenitor Cells after 72hrs exposure with compounds

Figure 2: Heat Map of cell viability after 72hrs exposure with compounds. Two different StemoniX NPC lines (derived from two different individuals) were exposed to 5 concentrations of compounds (10 - 0.001µM). PrestoBlue (Thermo) and CellTiter-Glo (Promega) were used to assess viability. Heat map units in percentage of viability.

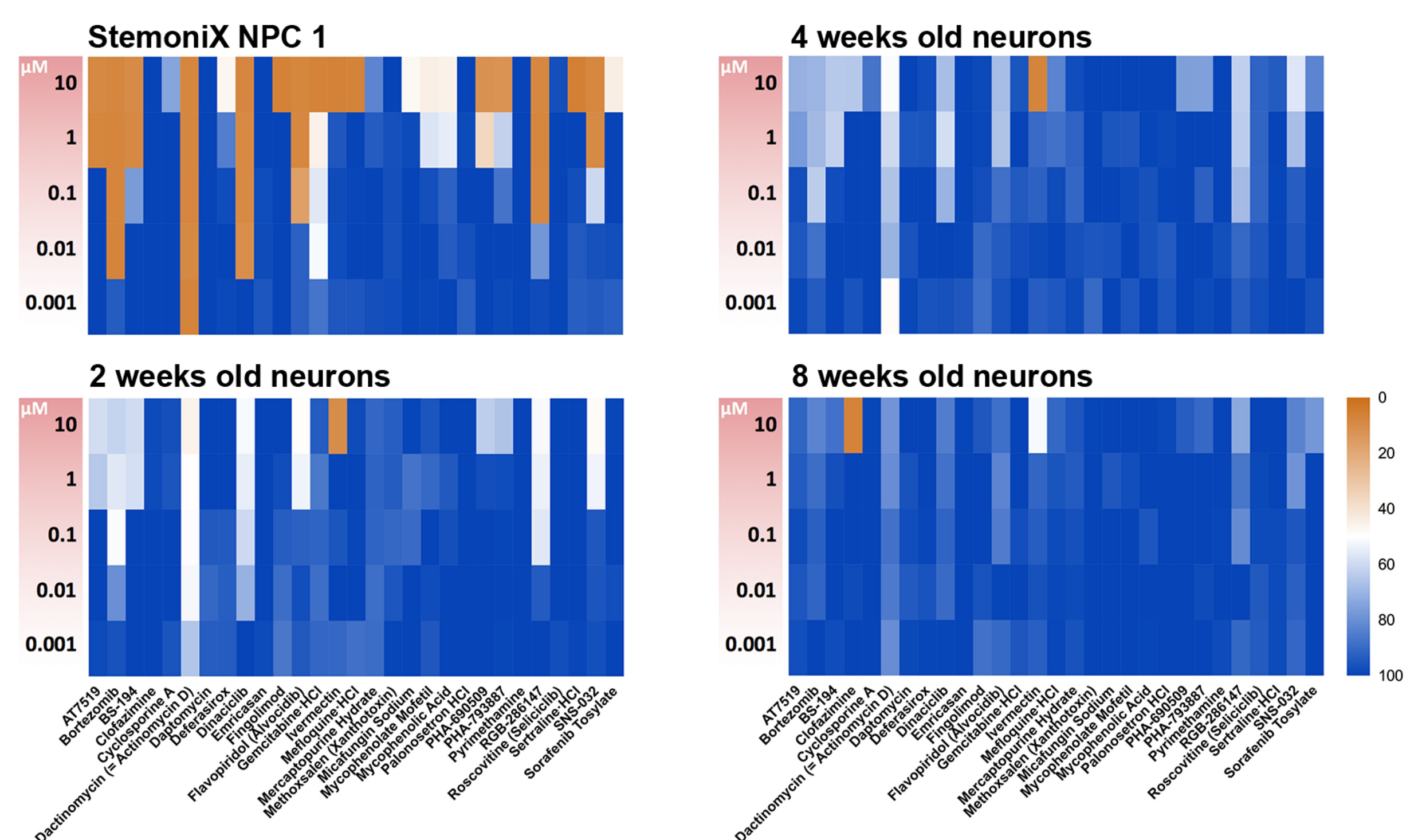### Cell Viability at different stages of neuronal development

Figure 3: Heat Map of cell viability at different stages of cortical neuronal differentiation. PrestoBlue (Thermo) was used to assess cell viability. Heat map units in percentage of viability.
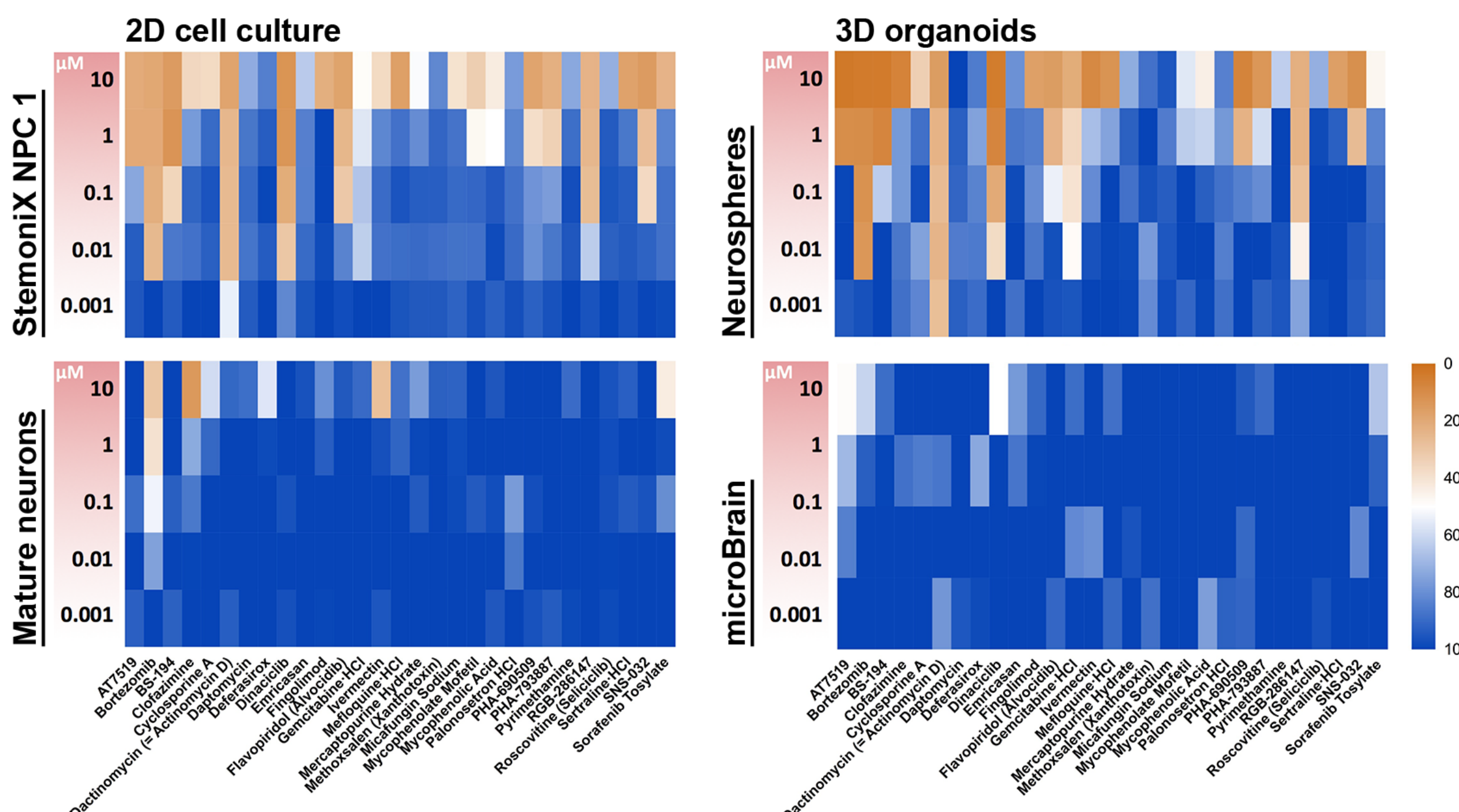
Cell Viability in 3D organoids culture

Figure 4: Heat Map of cell viability in 3D organoids culture compared to traditional (2D) cell culture. CellTiter-Glo 3D (Promega) was used to assess cell viability. Organoids challenged in 2 different stages: Neurospheres (progenitor) and microBrain (mature neurons). Heat map units in percentage of viability.
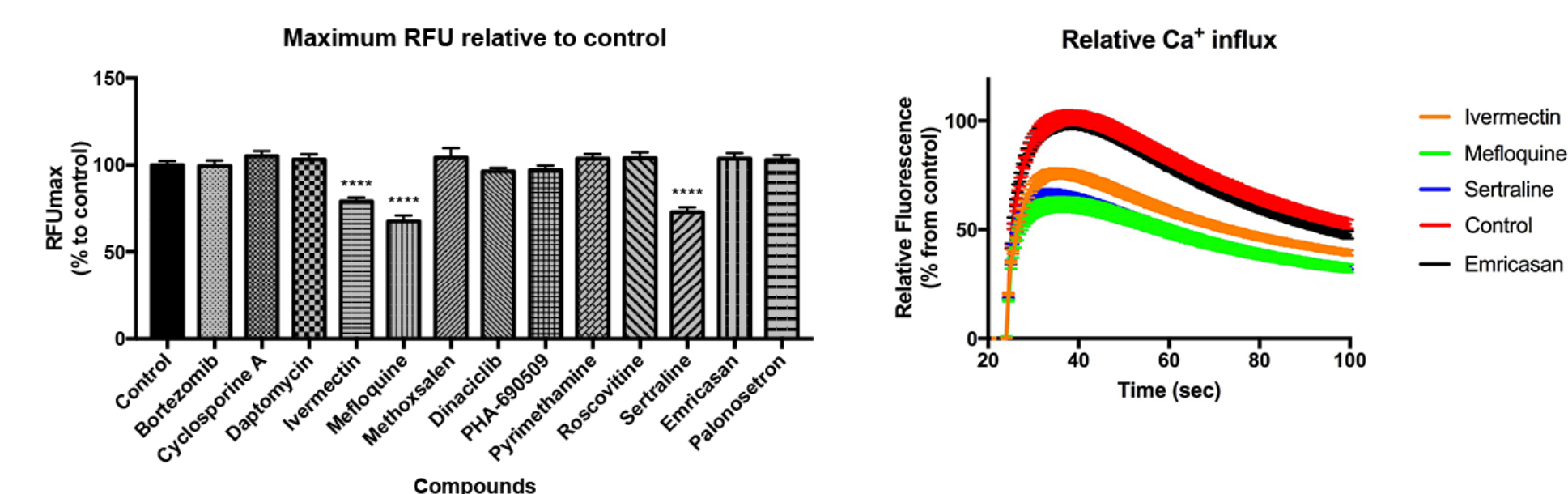
Ca+ mobilization assay on mature neurons
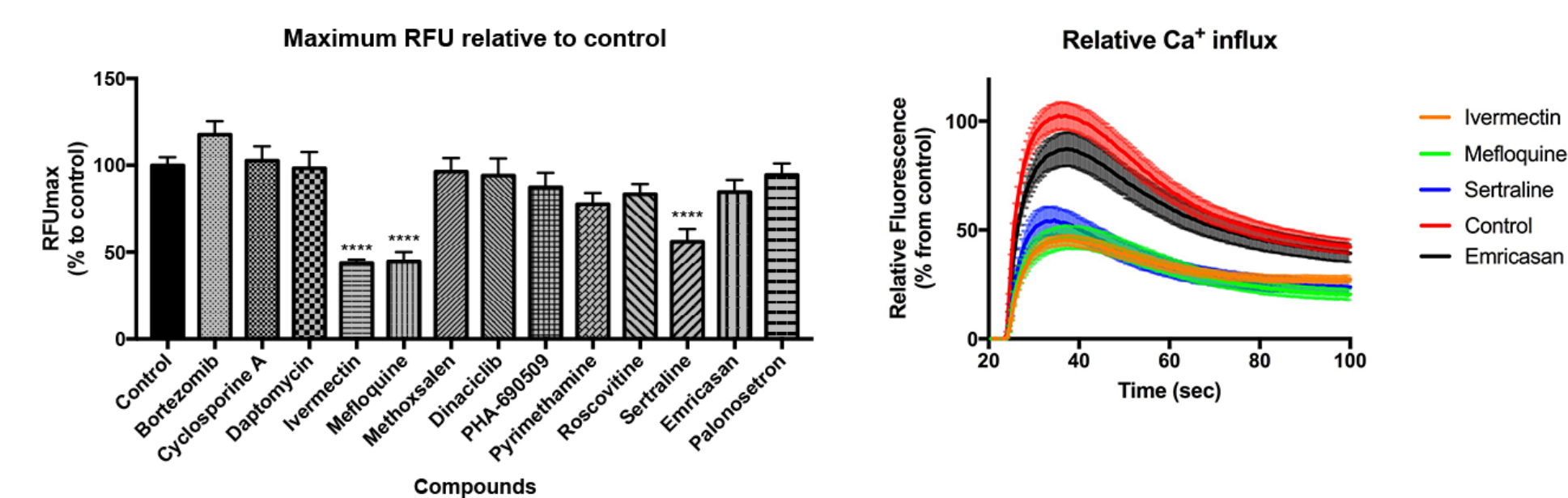
Figure 5: Ca+ mobilization assays on mature neural cultures after exposure to compounds. Cells were exposed to compounds for 2hrs before challenging with either Glutamate (200µM) or Kainic Acid (100µM) and fluorescence was measured using the FLIPR[Tetra] system (Molecular Devices). Compound concentration: 10µM. **** represents $p < 0.0001$ compared to Relative Fluorescent Units (RFU) from control (Glutamate or Kainic Acid).
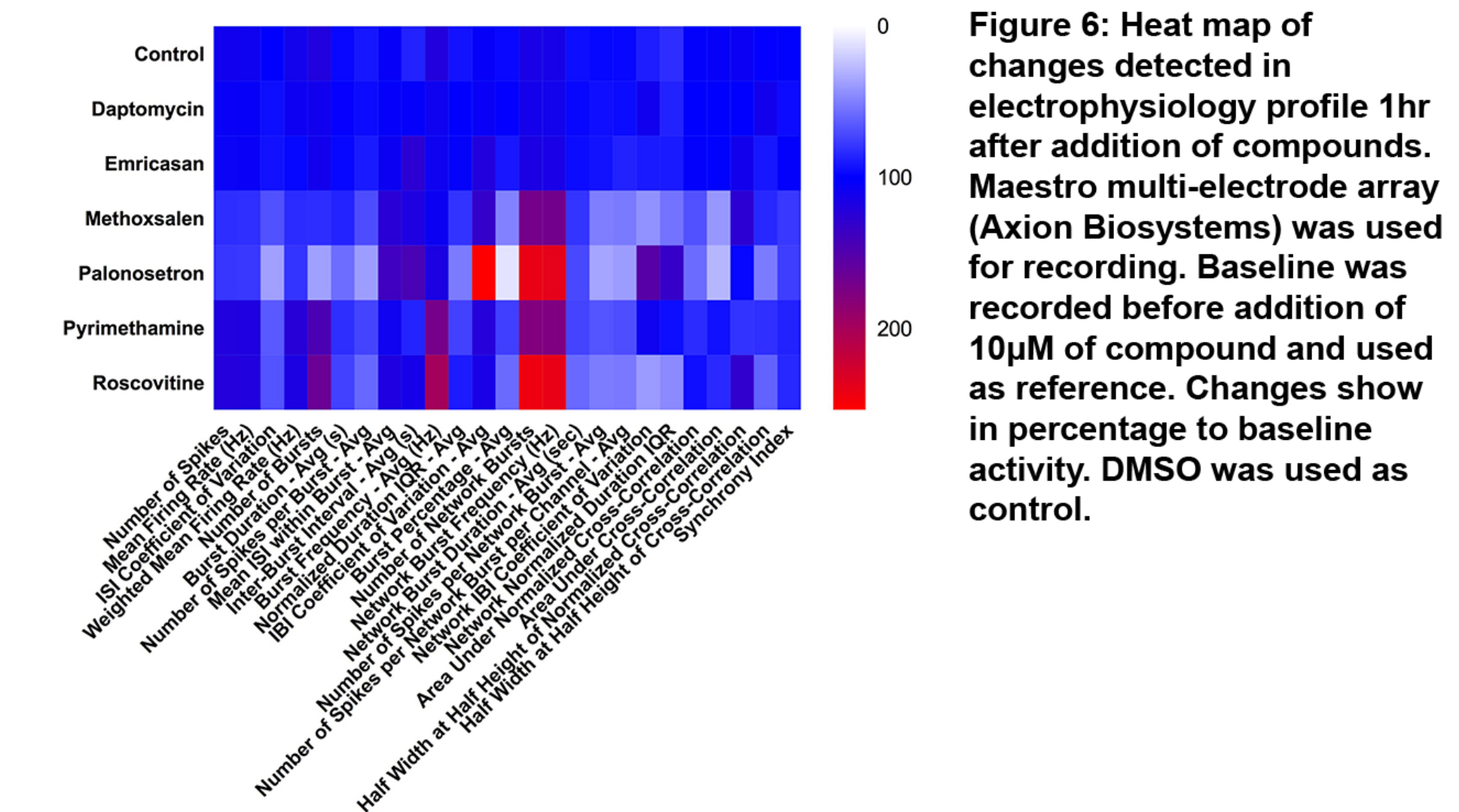
MEA electrophysiology assay on mature neurons

Figure 6: Heat map of changes detected in electrophysiology profile 1hr after addition of compounds. Maestro multi-electrode array (Axion Biosystems) was used for recording. Baseline was recorded before addition of 10µM of compound and used as reference. Changes show in percentage to baseline activity. DMSO was used as control.

Conclusions
Assessing the toxicological profile of chemical entities is critical for the successful development of new drugs. Even already approved compounds need to have their safety investigated when repurposed to new diseases, especially if targeting a new tissue. Using a human iPSC-based screening platform, we investigated the safety profile of 29 compounds recently described as effective against the Zika Virus infection. We observed great susceptibility to compound toxicity of the neural cultures at early stages of development, while toxicity decreased as the neuronal cultures matured *in vitro*. Although many compounds displayed toxicity in early stages of neural development, a few compounds showed reduced or no toxicity at high dosages. Emricasan, a compound highlighted in a previous publication for its effectiveness against Zika Virus, exhibited a safe toxicological profile in all neural stages of our screening. On the other hand, neural progenitor cells were highly sensitive to CDK inhibitors, clearly demonstrating the importance of the cell cycle on these cells. A deleterious effect from toxic compounds on these cells could result in a catastrophic outcome for a developing organism and more studies are necessary before their prescription to pregnant women. The present work shows the value of a human screening system that is able to mimic both early and late stages of nervous system development. This system can be applied to understand the safety profiles on new chemical entities, improve predictability of clinical outcomes and reduce overall drug development costs.