

Optogenetic technologies enable high throughput ion channel drug discovery and toxicity screening

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Abstract

The drug discovery process involving ion channels needs to rely on high-throughput screening (HTS) assays as well as fine-tuned characterization by electrophysiological measurements. Combining optogenetic tools with induced pluripotent stem-cells (iPSC)-derived cardiomyocytes can provide a new reliable, cost-effective and highly time-resolved approach to induce electrogenic proteins activation. The aim of the OPTEL project, which combines the expertise of two leading European companies and one outstanding academic laboratory, was to develop integrated HTS-compatible platforms based on optogenetic tools (Channelrhodopsin, ChR2) for effective drug discovery in heterologous expression systems and iPSC-derived cardiomyocytes. We generated HEK293 cell lines stably expressing ChR2 alone or with the cardiac sodium channel, Nav1.5 which proper functional expression was validated by fluorescence and manual patch-clamp. The best clones were used to implement light stimulation on an HTS automated patch-clamp platform (SynchroPatch 384PE, Nanion Technologies). We recorded light-induced currents and action potential-like responses in voltage and current-clamp, respectively, validating a new platform for high-throughput automated “opto-patch-clamp” assays. For optogenetic drug toxicity screening, commercially available iPSC-derived cardiomyocytes were infected with adeno-associated viruses to express ChR2 and analyzed by recording extracellular field potentials from 96 wells with a modified CardioExcyte96 system (Nanion Technologies). This was equipped with a custom designed 96 LED lid enabling pacing of cells and frequency-dependent drug screening over the physiological heart rate (1-3 Hz). Thereby we characterized adverse side effects of known ion channel blockers and pro-arrhythmogenic drugs on Na⁺, Ca²⁺ and K⁺ channel function. These results validate new platforms that allow optogenetic control of electrical activity in HTS-compatible format with four different readouts: fluorescence, electrophysiology, impedance and EFP. The use of these complementary readouts provides a new cost-effective and informative strategy in early stages of ion channel drug discovery, especially in the cardiac field.

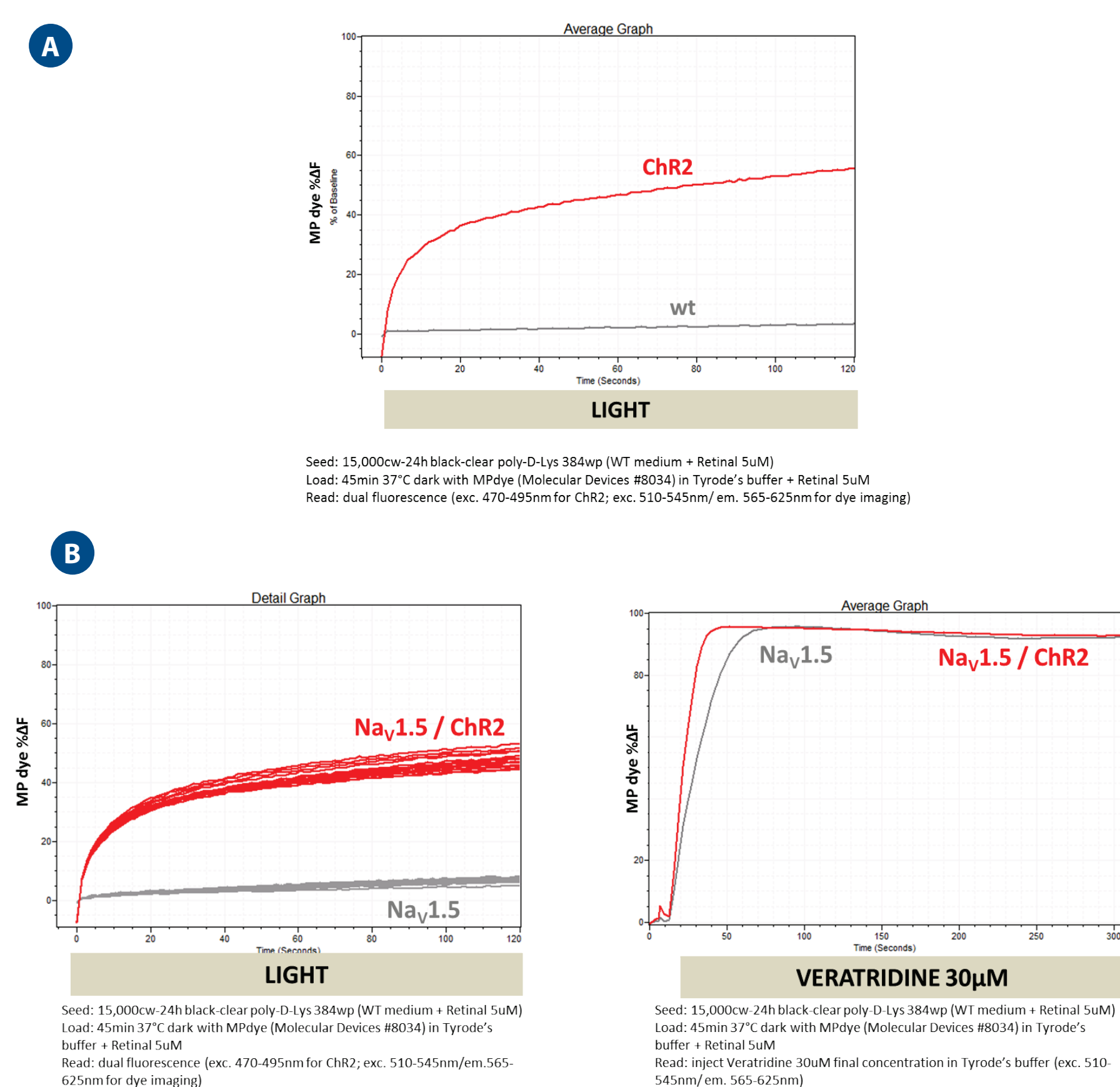
Introduction

The recent advances in the optogenetic field allow the possibility to use the light stimulation of Channelrhodopsin as appealing activating tools for in-vitro drug screening and toxicity tests. Another major advance in the first phases of drug discovery process, especially in the cardiac field, is the growing availability of iPSC-derived cardiomyocytes; however, intrinsic automaticity of these cells requires still a fast, reproducible and clean electrical pacing to perform reliable drug characterization. The general aim of this project was to combining the existing technologies with optogenetics and iPSC derived cardiomyocytes to develop an integrated platform for new reliable high-throughput screening strategies, taking advance of the optical control of membrane fluxes and/or depolarization via channelrhodospin activation. Four commonly used readouts were assessed: fluorescence, electrophysiology, impedance and EFP.

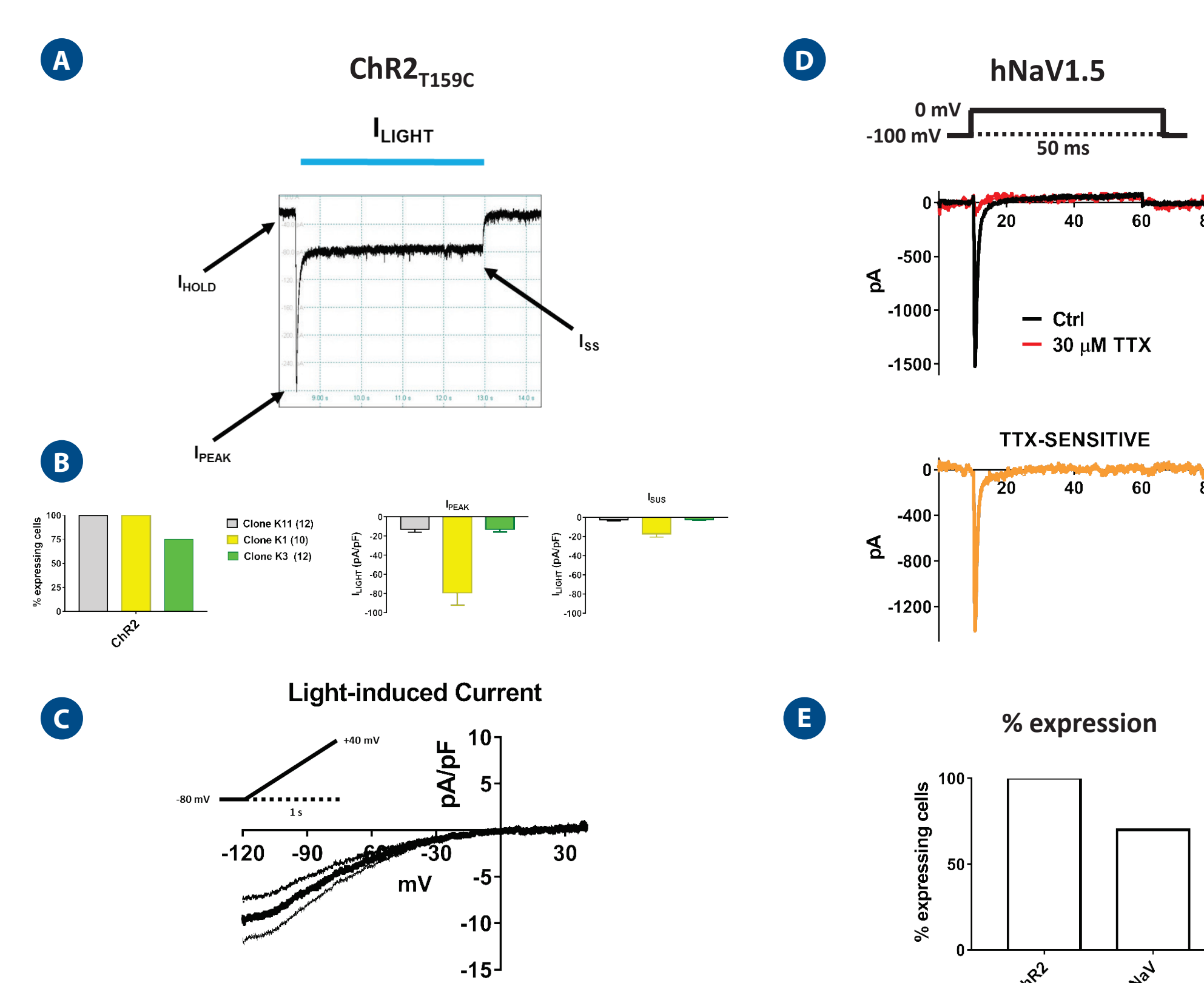
Cell line validation

A. HEK293 cells stably transfected with ChR2_{T159C} and their respective mock control (WT) were analyzed at FLIPR^{TETRA} with membrane potential sensitive dye. The panel shows a representative fluorescence trace from the best performing clone expressing ChR2_{T159C} and a WT (red and black lines, respectively) during repetitive light stimulation.

B. Following the final clone purification, the best performing HEK293/ChR2_{T159C} clone was stably transfected with hNav1.5 and analyzed at FLIPR^{TETRA}, along with their mock counterparts with membrane potential sensitive dye. The panels illustrate representative fluorescence traces obtained either by light stimulation or exposure to veratridine (left and right panels, respectively).



Electrophysiology validation



light induced currents evoked by blue light during a slow voltage ramp on the final selected clone (K1). The voltage protocol is shown in the inset. N=7.

D. Representative traces of currents evoked by a 50 ms-long voltage step from -80 mV (Holding) to 0 mV in the best performing clone of the HEK293/ChR2_{T159C}/NaV1.5 cell line. 30 μ M TTX was used to univocally isolate the NaV1.5-dependent currents. The lower panel shows the TTX-sensitive current obtained by digital subtraction of the traces in the upper panel. Similar results were obtained in 8 cells.

E. Percentage of cells expressing ChR2_{T159C} and NaV1.5 in the best performing clone. Presence and functionality of ChR2_{T159C} were confirmed using the experimental conditions described on panel A.

Optical Automated Electrophysiology

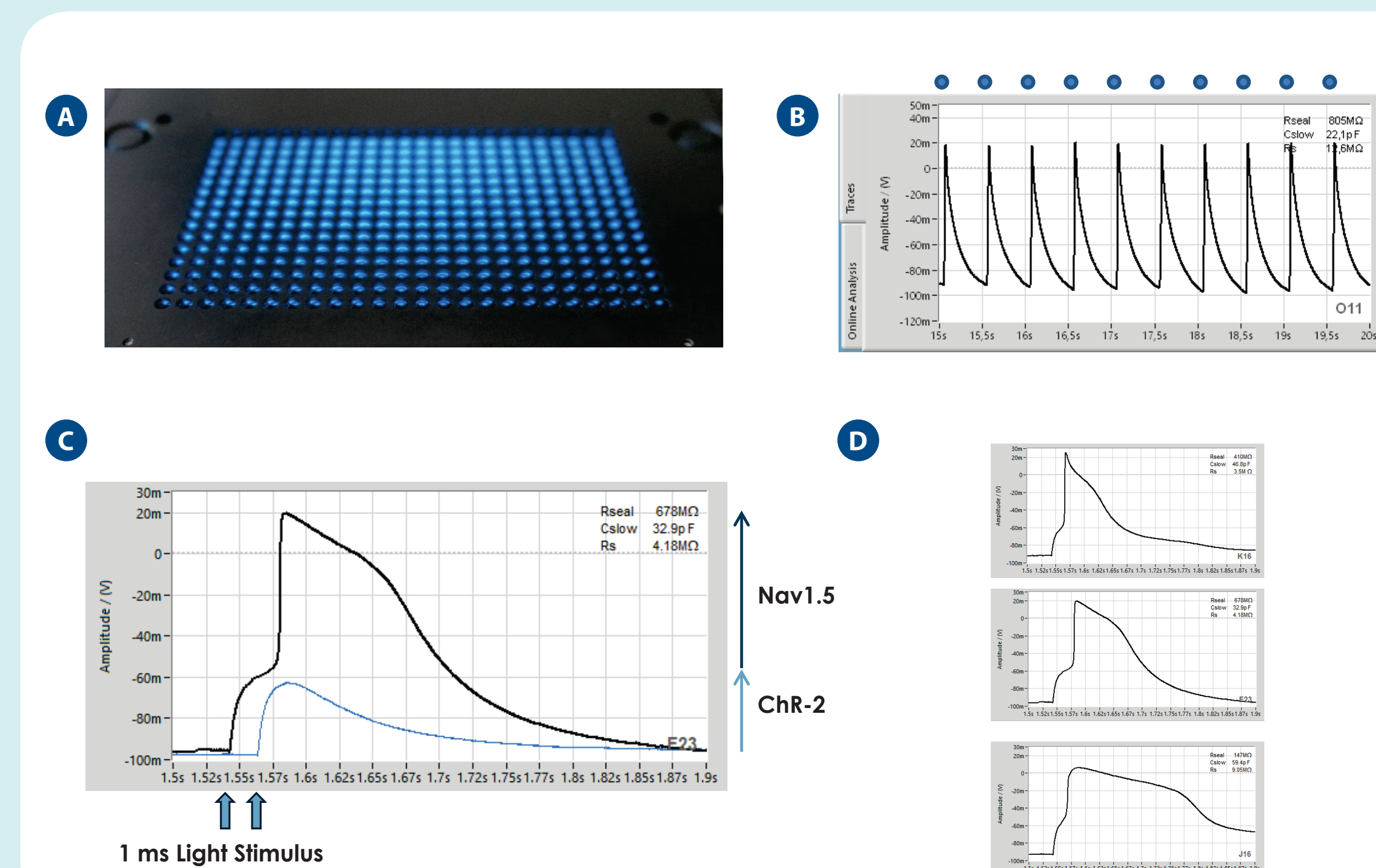
These figures show some ideas how optical stimulation of blue light (470 nm) with planar patch clamp could be combined. A panel of 96 LED is built below the measuring chamber of a SynchroPatch 384PE. This allows the simultaneous application of light stimuli with 384 parallel electrophysiological recordings.

A. Shows a picture of the blue light used for stimulation within the SynchroPatch 384PE.

B. HEK293/ChR2_{T159C} cells were used in this experiment. The cells were measured in Current Clamp mode using the SynchroPatch 384PE. A Resting voltage of -90 mV was achieved by LFVC (Low Frequency Voltage Clamp) to close the NaV1.5 channels. Light pulses of 1 ms at 2Hz (470 nm) were used for stimulation. The NaV1.5 channels opened due to the depolarization by ChR2_{T159C}.

C. Cor4U iPSC derived cardiomyocytes transfected with ChR2_{H134R} were used in this experiment. The cells were measured in Current Clamp mode using the SynchroPatch 384PE. A Resting voltage of -90 mV was achieved by LFVC (Low Frequency Voltage Clamp). Light pulses of 1 ms at 0.5Hz (470 nm) were used for stimulation. The depolarization by ChR2_{H134R} was able to elicit action potential.

D. This panel illustrates different examples of this experiments displaying various shapes of cardiac action potentials.



Opto-pacing of iPSC-derived cardiomyocytes

Optogenetically paced field potentials (FP) at various physiological stimulation rates.

A. Representative FP traces during (from left to right) spontaneous beating and optical pacing (blue bars) at 1 Hz, 2 Hz and 3 Hz.

B. Overlay of representative FP paced at frequencies 1–3 Hz (from black to light blue).

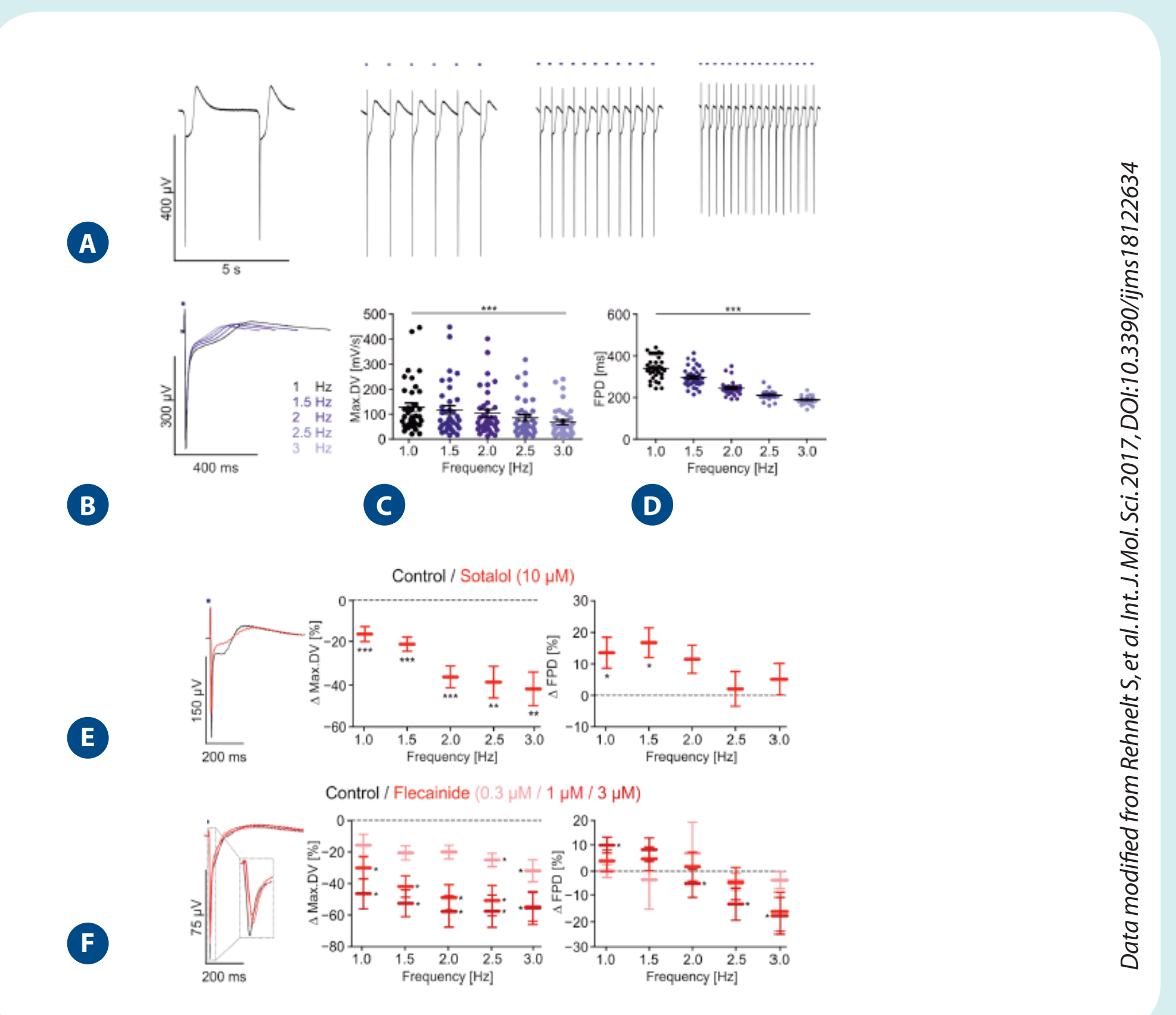
C. Statistical analysis of absolute values of max.dV (N=37).

D. Statistical analysis of field potential duration during pacing with indicated frequencies (FPD, N=42).

E, F. Overlay of representative FP optical paced at 1 Hz (left) before (black) and after drug application (red), and statistical analysis of the relative changes of max. DV (middle) and FPD (right) at the frequencies 1–3 Hz.

E Effects of 10 μ M Sotalol (n = 13–17);

F Effects of 0.3, 1 and 3 μ M Flecainide (n = 11–12). *p < 0.05, **p < 0.01, ***p < 0.001.



Conclusions

Within the consortium, we generated a pure cell line heterologously expressing both ChR2 and Nav1.5 whose functionality was validated on the state-of-the-art manual patch-clamp; on another hand we develop HTS-compatible platforms based on optogenetic tools for effective drug discovery in heterologous expression systems and iPSC-derived cardiomyocytes. Taken together, the present results illustrate a scalable approach for preclinical screening of drug effects on cardiac electrophysiology. Importantly, we show that the recording and analysis can be fully automated and the technology is readily available using commercial products.

Acknowledgements
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