

# The Na<sub>v</sub>1.5 Late Current in WT and Na<sub>v</sub>1.5-ΔKPQ Mutant Channels: An Automated Patch Clamp LQT3 Electrophysiological Assay Comparison

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## Introduction

The cardiac late Na<sup>+</sup> current (late I<sub>Na</sub>) generates persistent inward currents throughout the plateau phase of the ventricular action potential and is an important determinant of repolarisation rate, EADs and arrhythmia risk<sup>1</sup>. As inhibition of late I<sub>Na</sub> can offset drug effects on hERG and other repolarising K<sup>+</sup> conductances it is one of the key cardiac channels in the Comprehensive *in vitro* Pro-arrhythmia Assay (CiPA) panel being developed by the FDA to improve human clinical arrhythmia risk assessment<sup>2,3</sup>. The standard CiPA late I<sub>Na</sub> assay uses the anemone toxin ATX-II to pharmacologically inhibit inactivation and produce persistent openings of wildtype (WT) Na<sub>v</sub>1.5 channels, but this method is variable and non-physiological. In contrast, several mutations in the SCN5A gene cause a form of hereditary long QT syndrome (LQT3) by promoting late openings<sup>4</sup>. The ΔKPQ mutation deletes residues Lys 1505, Pro 1506 and Gln 1507 and results in a sustained, non-inactivating current during long depolarizations which causes prolongation of the action potential and can result in fatal ventricular arrhythmias such as Torsade de Pointes (TdP)<sup>5,6</sup>.

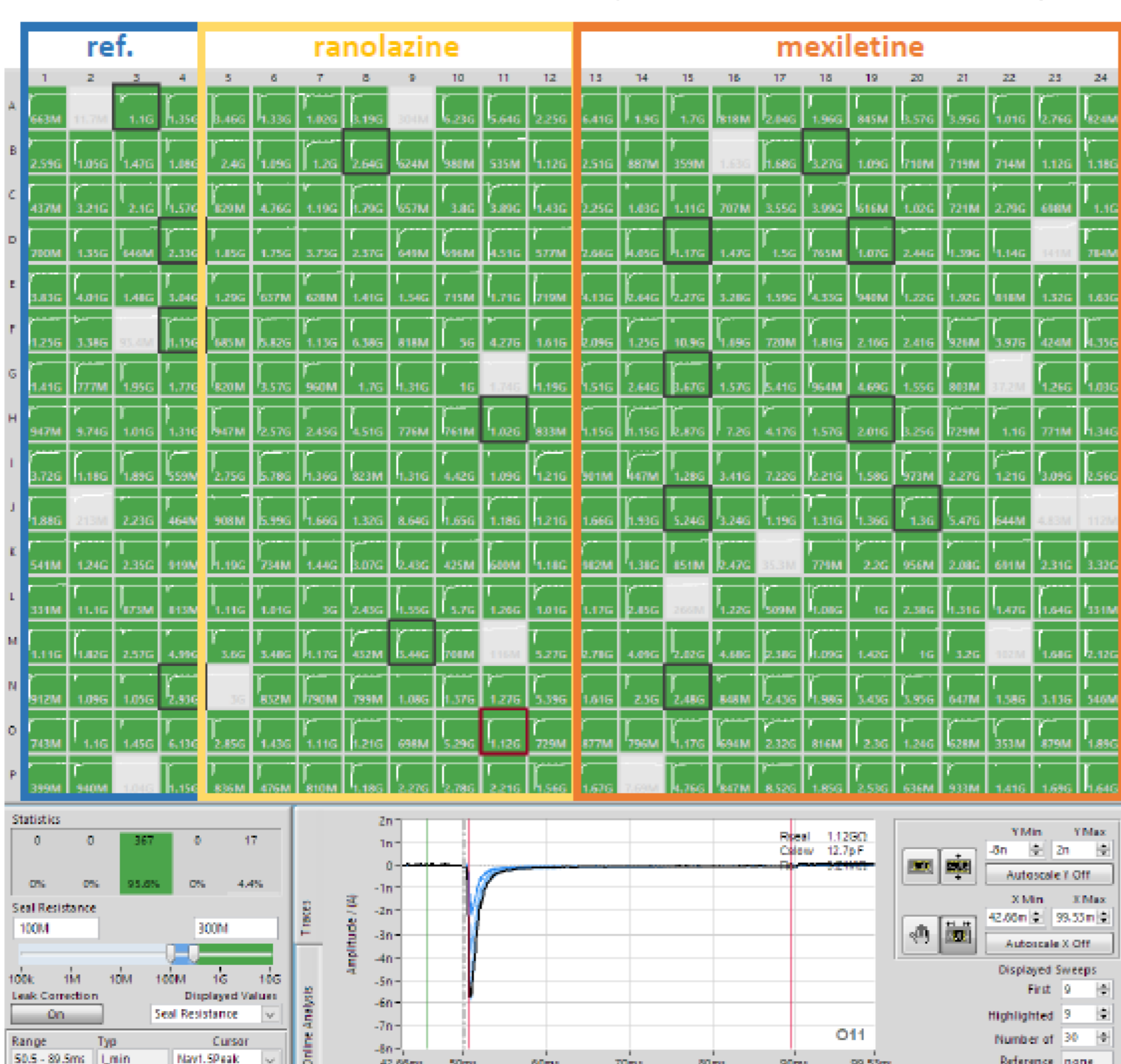
Here we utilised a stable Na<sub>v</sub>1.5 LQT3 mutant cell line to optimise and validate a high throughput automated patch clamp late I<sub>Na</sub> assay on the SyncroPatch 384i platform. High quality gigaseal recordings were obtained with a high success rate, enabling the efficient and accurate determination of relevant biophysical and pharmacological properties of this CiPA-compliant late Na<sub>v</sub>1.5 assay. The combination of the SyncroPatch 384i automated patch clamp system and Na<sub>v</sub>1.5 ΔKPQ cell line created a reliable high throughput cardiac safety screening assay without the need for openers like ATX-II toxin.

## Materials and methods

CHO cells expressing WT human Na<sub>v</sub>1.5 gene and HEK cells expressing Na<sub>v</sub>1.5 ΔKPQ mutant channel proteins were cultured and harvested according to standard protocols. Dissociated cell suspensions were kept at 10°C in the onboard cell hotel and dispensed onto NPC patch chips immediately prior to conventional whole-cell patch clamp recordings using standard sealing and patching protocols on the SyncroPatch384i platform, with minor adjustments to obtain a high proportion of gigaohm seals and stable late I<sub>Na</sub> current amplitude and kinetics. Intracellular solution was CsF-based and external contained 140 NaCl. Peak and late I<sub>Na</sub> currents were measured using a CiPA step-ramp voltage protocol for pharmacology screening, whilst voltage step protocols were used to determine activation and inactivation biophysical parameters, with Rs compensation enabled. Single-hole and multi-hole NPC plates were employed to assess and optimise late I<sub>Na</sub> current amplitudes. Test compounds for cumulative IC<sub>50</sub> screening were made up in 384 well plates at and dispensed by Biomek standard tips into each well (final 0.1% DMSO). Data was acquired Nanions' Patch Control Software with leak subtraction turned on and analysed and plotted using Nanions' DataControl software.

## 1. SyncroPatch384i APC ΔKPQ Na<sub>v</sub>1.5 assay

Very little optimisation of standard Na<sub>v</sub>1.5 cell line preparation and SyncroPatch384i APC assay conditions was required to achieve acceptable success rates for peak I<sub>Na</sub> recordings, as measured by sealing and patchability QC parameters and current expression levels (Fig. 1). In contrast, resolving inward late currents in the ΔKPQ cell line was more challenging but a ~50% QC success rate was achieved using 4x multi-hole chips (Table 1).



**Figure 1: Graphical interface plate view of ΔKPQ Na<sub>v</sub>1.5 peak current recording from 384 well single hole chip.** Wells exceeding minimum QC parameters (>300pA, >300 MΩ) are shown in green, and raw current traces from highlighted wells in 0.1% DMSO, Ranolazine and Mexiletine are shown below in trace view.

| Current type | Chip type | QC parameters |      | Success rate<br>% wells |
|--------------|-----------|---------------|------|-------------------------|
|              |           | pA            | MΩ   |                         |
| Peak ΔKPQ    | 1x high   | -300          | 300  | 95.6                    |
| Late ΔKPQ    | 1x high   | -200          | 1000 | 4.7                     |
| Late ΔKPQ    | 4x medium | -200          | 250  | 45.3                    |

**Table 1: Patchability, current expression and success rates for different Nav1.5 ΔKPQ assay conditions.** Comparison of assay performance obtained using single-hole vs 4x multi-hole NPC plates. Patchability QC parameters include whole-cell seal resistance, minimum current amplitude, and experimental success rate (completion of pharmacology screening or biophysical assessment over 20 min recording).

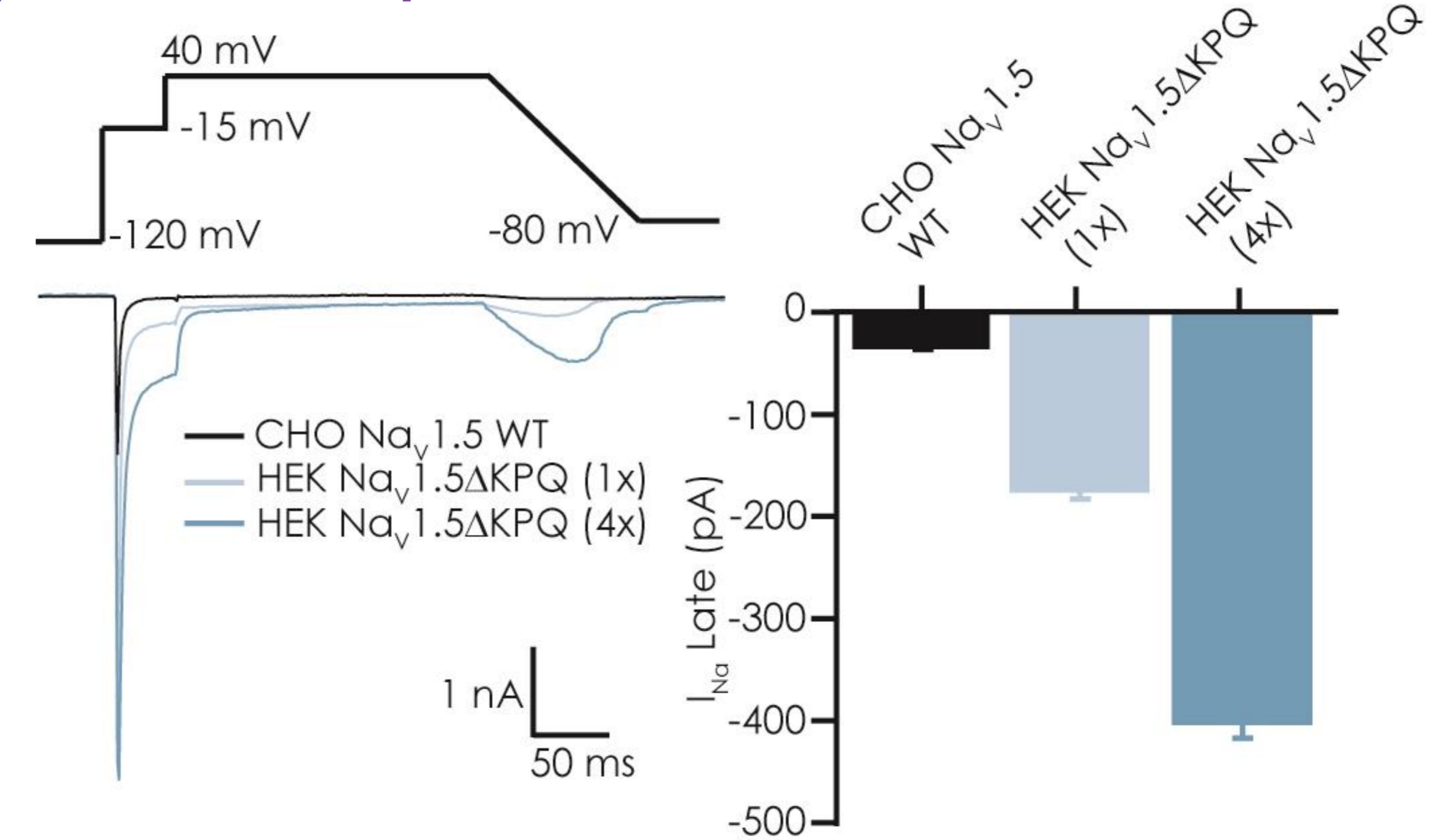
## References

- Wu *et al.*, (2006) An Increase in Late Sodium Current Potentiates the Proarrhythmic Activities of Low-Risk QT-Prolonging Drugs in Female Rabbit Hearts. *J Mol Pharm* 316: 718-726.
- Li *et al.*, (2018) Assessment of an In Silico Mechanistic Model for Proarrhythmia Risk Prediction Under the CiPA Initiative. *Clin Pharm Therapeutics*: 10.1002/cpt.1184.
- Crumb *et al.*, (2016) An evaluation of 30 clinical drugs against the comprehensive *in vitro* proarrhythmia assay (CiPA) proposed ion channel panel. *J Pharmacol Toxicol Methods*, 81: 251-262.
- Wang *et al.*, (1995) SCN5A Mutations Associated with an Inherited Cardiac Arrhythmia, Long QT Syndrome. *Cell* 80: 805-811.
- Wang *et al.*, (1996) Characterization of human cardiac Na<sup>+</sup> channel mutations in the congenital long QT syndrome. *PNAS* 93:13200-13205
- Bennett *et al.*, (1995). Molecular mechanism for an inherited cardiac arrhythmia. *Nature*. 376: 683-685.
- Guo & Jenkinson (2019). *J Pharm Tox Meth* 99: 106575.

## 2. ΔKPQ Na<sub>v</sub>1.5 current characterisation

A major challenge to recording late openings of Na<sub>v</sub>1.5 channels is their small amplitude, which is negligible in WT channels (Fig. 2) but resolvable in ΔKPQ mutant channels using single-hole APC chips and of sufficient size for reliable biophysical and pharmacological assessment using multi-hole chips.

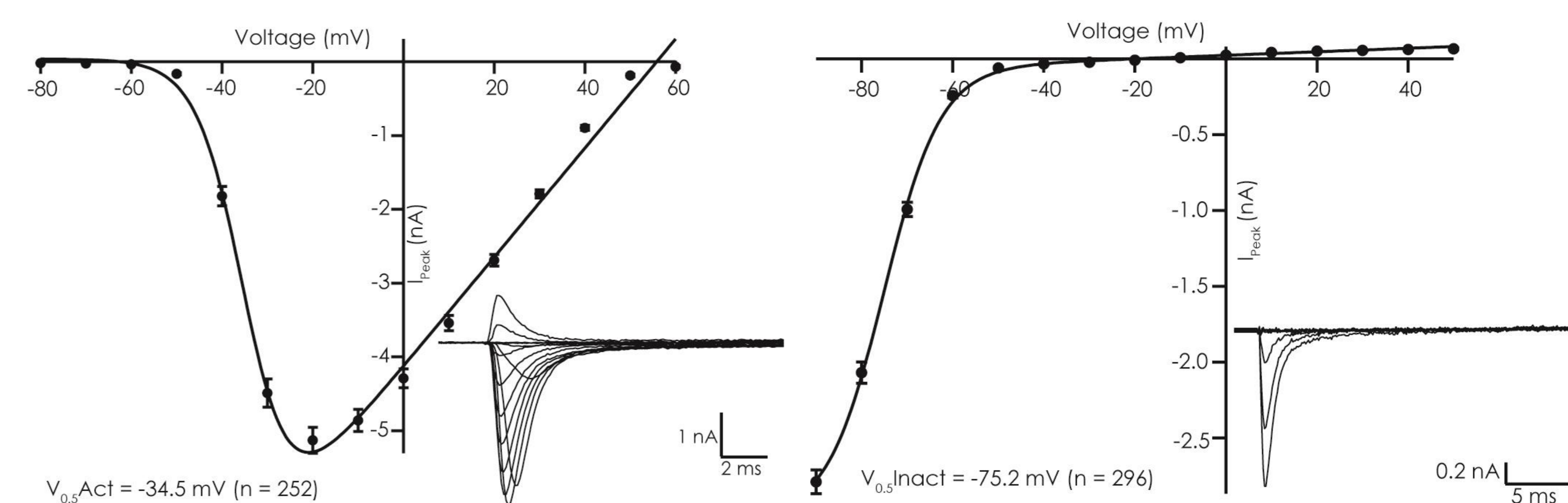
### Na<sub>v</sub>1.5 current expression



**Figure 2: Comparison of peak (left) and late (right) Na<sub>v</sub>1.5 current components in WT and ΔKPQ cell lines recorded using single- and multi-hole chips on SP384i using a CiPA step-ramp voltage protocol.**

LQT3 ΔKPQ Na<sub>v</sub>1.5 currents exhibited steady state voltage-dependent activation (V<sub>1/2</sub> -35 mV, left shifted compared to WT of -15 mV) and inactivation properties (V<sub>h1/2</sub> -75 mV) as expected using standard protocols<sup>5,6</sup>.

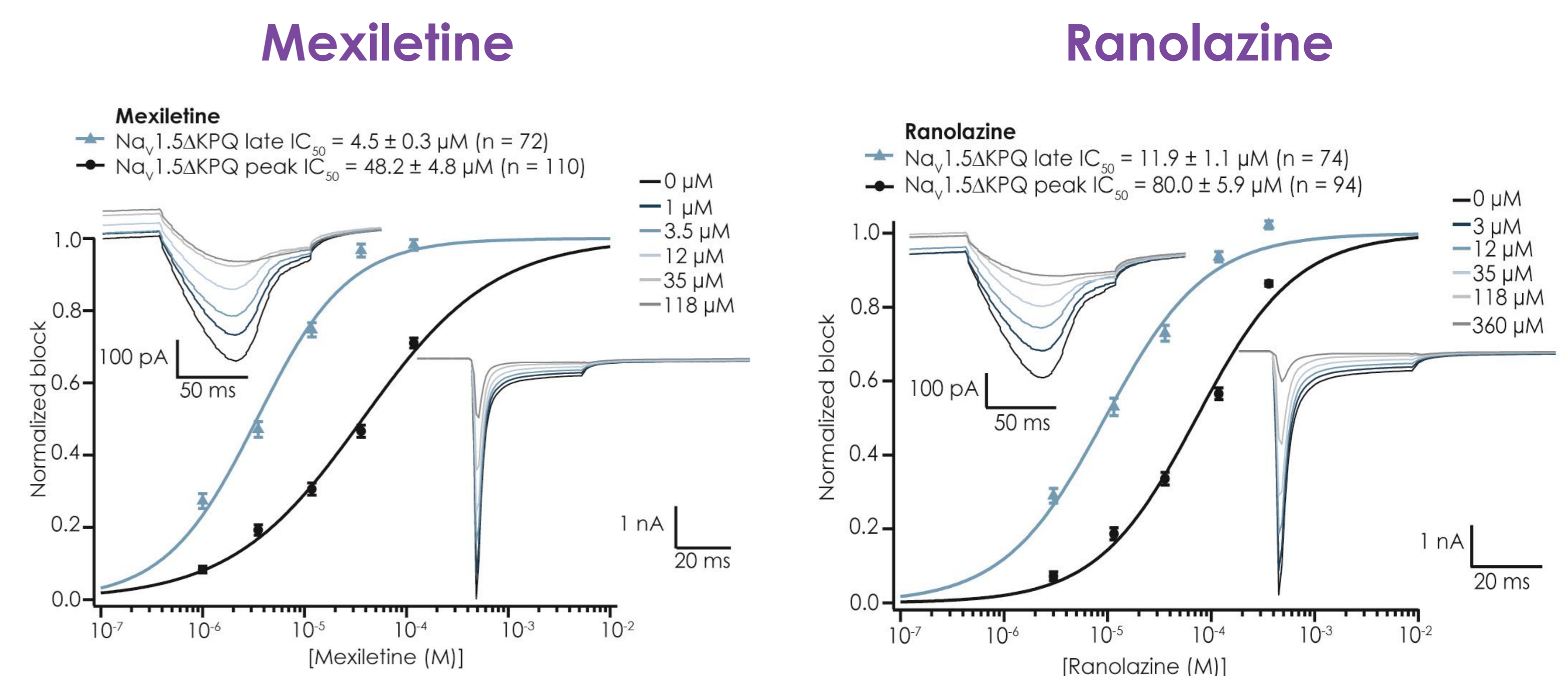
### Na<sub>v</sub>1.5 biophysics



**Figure 3: Voltage-dependent biophysical properties of ΔKPQ peak Na<sub>v</sub>1.5 currents.** Activation (left) and steady-state inactivation (right) were determined using voltage steps delivered from a V<sub>h</sub> of -100 mV.

## 3. Reference pharmacology

As there is little HTS APC data on ΔKPQ LQT3 late I<sub>Na</sub> pharmacology it was important to test reference compounds and compare their potency to non-physiological ATX-II toxin-activated channels, using the CiPA step-ramp protocol. We found that Ranolazine and Mexiletine inhibited mutant late I<sub>Na</sub> with an IC<sub>50</sub> of 17.5 μM (n = 74 wells) and 6.4 μM (n = 73 wells), respectively (Fig. 4), in good agreement with published values<sup>7</sup>. Peak WT and ΔKPQ currents were less sensitive than late I<sub>Na</sub> components (Table 2).



**Figure 4: Pharmacological validation of ΔKPQ Na<sub>v</sub>1.5 currents.** The inhibition of peak (black circles) and late current (blue triangles) is plotted against applied concentration of Mexiletine (left) and Ranolazine (right), and mean IC<sub>50</sub> values are shown above each figure. Inset into each figure are late currents (upper left) evoked at the end of a step-ramp protocol, as well as peak and non-inactivating currents evoked using a step protocol, from 4x multi-hole chip recordings; note different amplitude axis range.

| Compound   | CHO WT Na <sub>v</sub> 1.5 |      | HEK ΔKPQ Na <sub>v</sub> 1.5 (1x) |      | HEK ΔKPQ Na <sub>v</sub> 1.5 (4x) |      | Literature* |      |
|------------|----------------------------|------|-----------------------------------|------|-----------------------------------|------|-------------|------|
|            | Peak                       | Late | Peak                              | Late | Peak                              | Late | Peak        | Late |
| Ranolazine | 143                        | -    | 46.2                              | 12.3 | 80.0                              | 11.9 | 79.5        | 16.7 |
| Mexiletine | 83                         | -    | 17.1                              | 4.7  | 48.2                              | 4.5  | 21.9        | 12.2 |

**Table 2: Comparison of reference compound inhibition (IC<sub>50</sub>, μM) of peak vs late Na<sub>v</sub>1.5 currents.** Mexiletine was a more potent inhibitor of Na<sub>v</sub>1.5 currents than Ranolazine, and both compounds exhibited a 4-6 fold preference for the late I<sub>Na</sub> current component of ΔKPQ channels. \*Potency and selectivity for late openings were similar to that in published literature using ATX-II on WT Na<sub>v</sub>1.5 currents.

## Conclusions

- Our collaboration was successful in using Metrion's WT and ΔKPQ Na<sub>v</sub>1.5 cell lines and Nanion's SyncroPatch 384i APC platform to design, optimise and validate a CiPA-ready HTS cardiac safety screening assay suitable for prediction of human clinical pro-arrhythmia risk.
- Gigaseal quality recordings were key to resolving the small late current openings of LQT3 mutant Na<sub>v</sub>1.5 channels without resorting to use of ATX-II.
- Na<sub>v</sub>1.5 ΔKPQ biophysical and pharmacological properties were reliably and efficiently determined for this optimised APC HTS cardiac safety assay.

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