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High-throughput automated patch-clamp of lysosomal channel TRPML1 using Sophion Bioscience platforms

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Introduction

Traditional whole-cell patch clamp of lysosomes is laborious and extremely specialized, yet the lysosome contains a myriad of ion channel and transporter targets. Enhancing clearance of aggregates through lysosomal activation has proposed implications in neurodegenerative diseases such as Parkinson's Disease, and more recently ion channel specific rare genetic diseases. Sophion Bioscience has therefore developed a method for high-throughput automated patch clamp (APC) of enlarged lysosomes (Lysopreps provided by Oria Bioscience) using the Qube 384 and QPatch 48 platforms.

This APC method involves using small-hole, high-resistant consumables (QChip Custom and QPlate HiR) for the purpose of capturing organelles and forming tight seals which enable stable recordings of ion channels in their endogenous environment. We have demonstrated the ability to capture lysosomes with a success rate of 50-70% using physiological pH and without the use of seal enhancers such as fluoride in the internal solution. Further, we are able to record ion channel currents and concentration-response curves for TRPML1, suitable for high-throughput compound screening.

References

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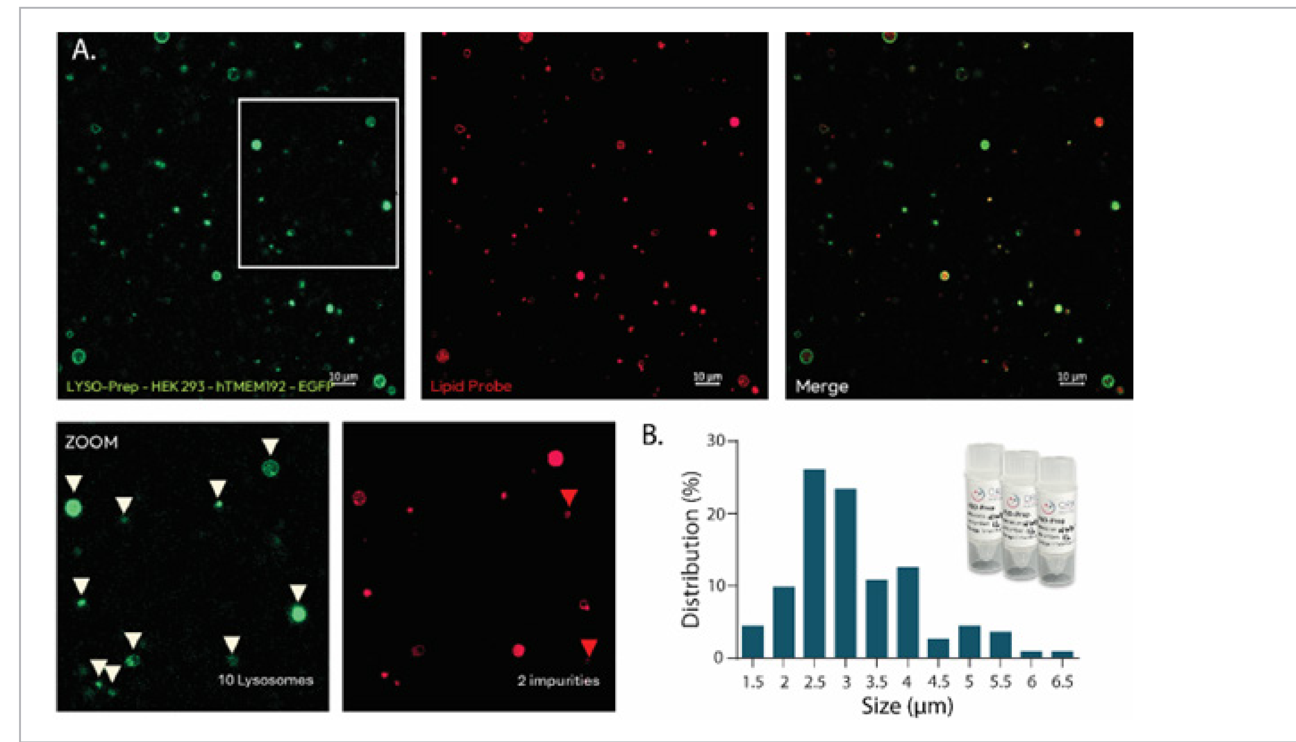

Fig. 1: LYSO-Prep™ from Oria Bioscience. (A) Confocal microscopy images of LYSO-Prep™ from HEK293 cells overexpressing hTMEM192-EGFP and labeled with a lipid probe (staining all membranes), illustrating the purity of the samples (63× oil objective). (B) Standard size distribution of LYSO-Prep™ following enlargement with 1 µM vacuolin, with an average size of approximately 3.16 µm. Only patchable lysosomes (> 1 µm) were analyzed using FIJI software.

Successful lysosome capture using small-hole consumables

First, we evaluated lysosome capture performance, meaning the ability to capture a lysosome on a patch hole and obtain a high resistance seal. For these experiments, we used LYSO-Prep™ lysosomes from Oria Bioscience, enlarged with 1 µM vacuolin to an average diameter of 3.16 µm (Figure 1). Using high-resistance (HiR) consumables with a reduced patch-hole diameter, the capture success rates were 69.9 ± 3.2% on the QPatch 48 (n = 10 plates) and 74.6 ± 2.6% on the Qube 384 (n = 10 plates; Figure 2), demonstrating robust performance across systems. All recordings were performed using fluoride-free seal-enhancing solutions, showing that stable seals can be achieved without relying on fluoride-based seal enhancers.

The use of HiR consumables also reduced "lysosome slipping", where lysosomes pass through the patch hole instead of forming a seal. This was evident from characteristic resistance peaks observed during positioning (Figure 3). In addition to improving capture reliability, the smaller patch hole increases the overall resistance, enhancing the resolution for detecting small currents (< 100 pA).

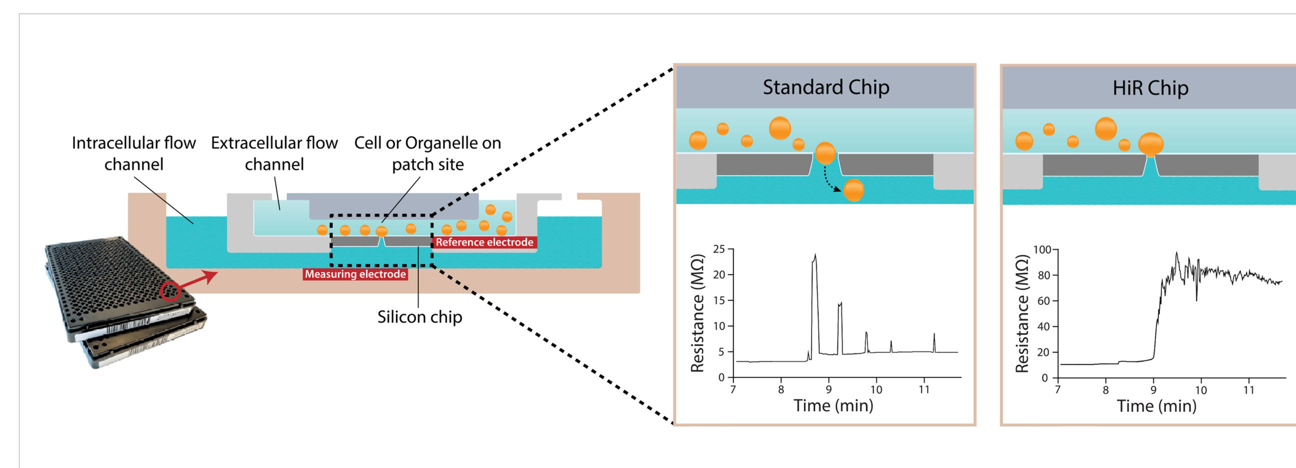

Fig. 2: High-resistance consumables reduce lysosome slipping. A cross-section illustration of a small-hole, high-resistance (HiR) consumable showing the reduction of lysosome slipping and successful lysosome capture. On a standard chip lysosome slipping can be observed on the resistance plot during lysosome capture, on a HiR chip this slipping is reduced, and lysosome capture can be confirmed by rapid and persistent increase in resistance.

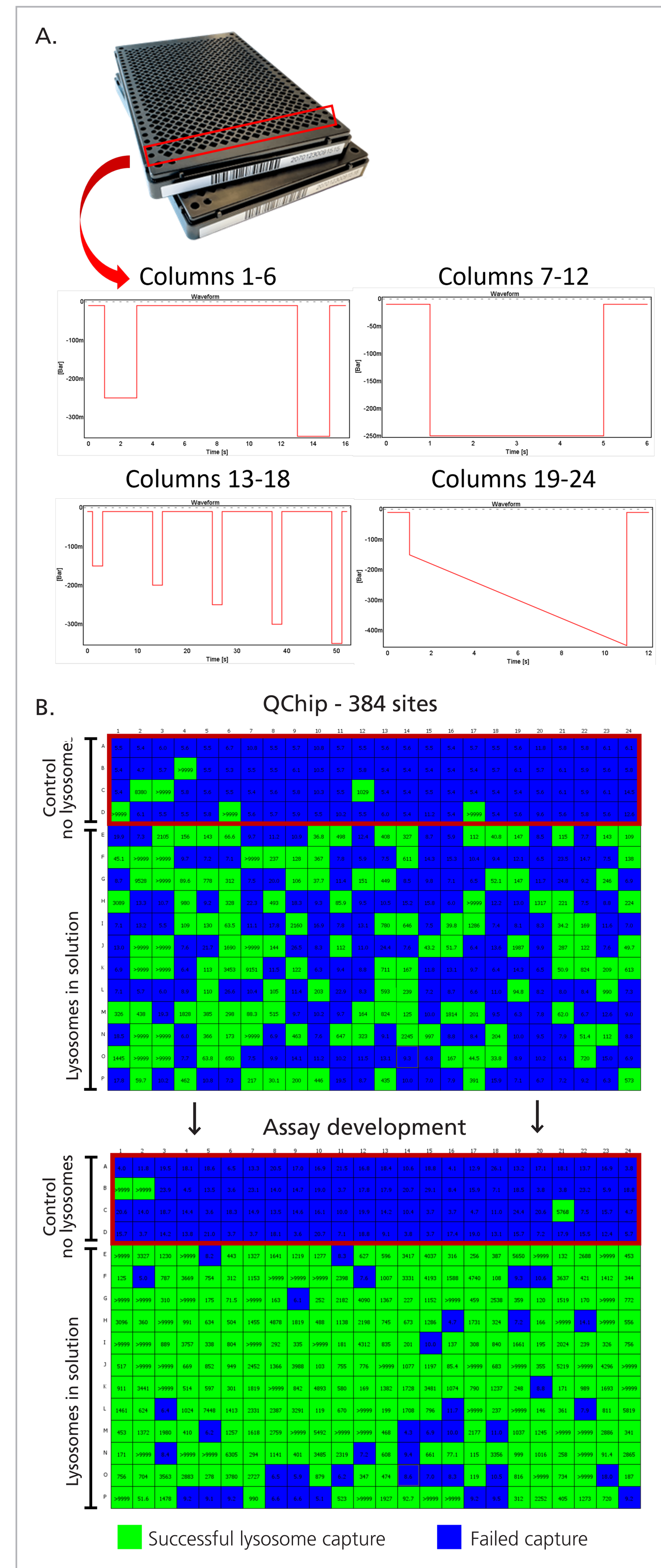

Fig. 3: Assay development increased successful lysosome capture rates. (A) Examples of differential whole-cell protocols used to optimize lysosome patching. Qube 384 plates can perform multiple different protocols across a single chip accelerating assay development. (B) Two example plate images from the Qube 384 illustrating the distribution of successful lysosome captures. The first 4 rows (A to D) were used as negative controls (no lysosomes in solution). Top: One of the first experiments with low lysosome capture rates. Bottom: Experiment after adjustment of lysosome capture protocols, solutions, and lysosome density. Successful sites are marked in green and unsuccessful sites in blue. Successful lysosome capture rates were 74.6 ± 2.6% on the Qube 384 (n = 10 plates) and 69.9 ± 3.2% on the QPatch 48 (n = 10 plates; plate images not shown). QC filters were applied (see Methods section).

Results

Pharmacological validation of TRPML1 current

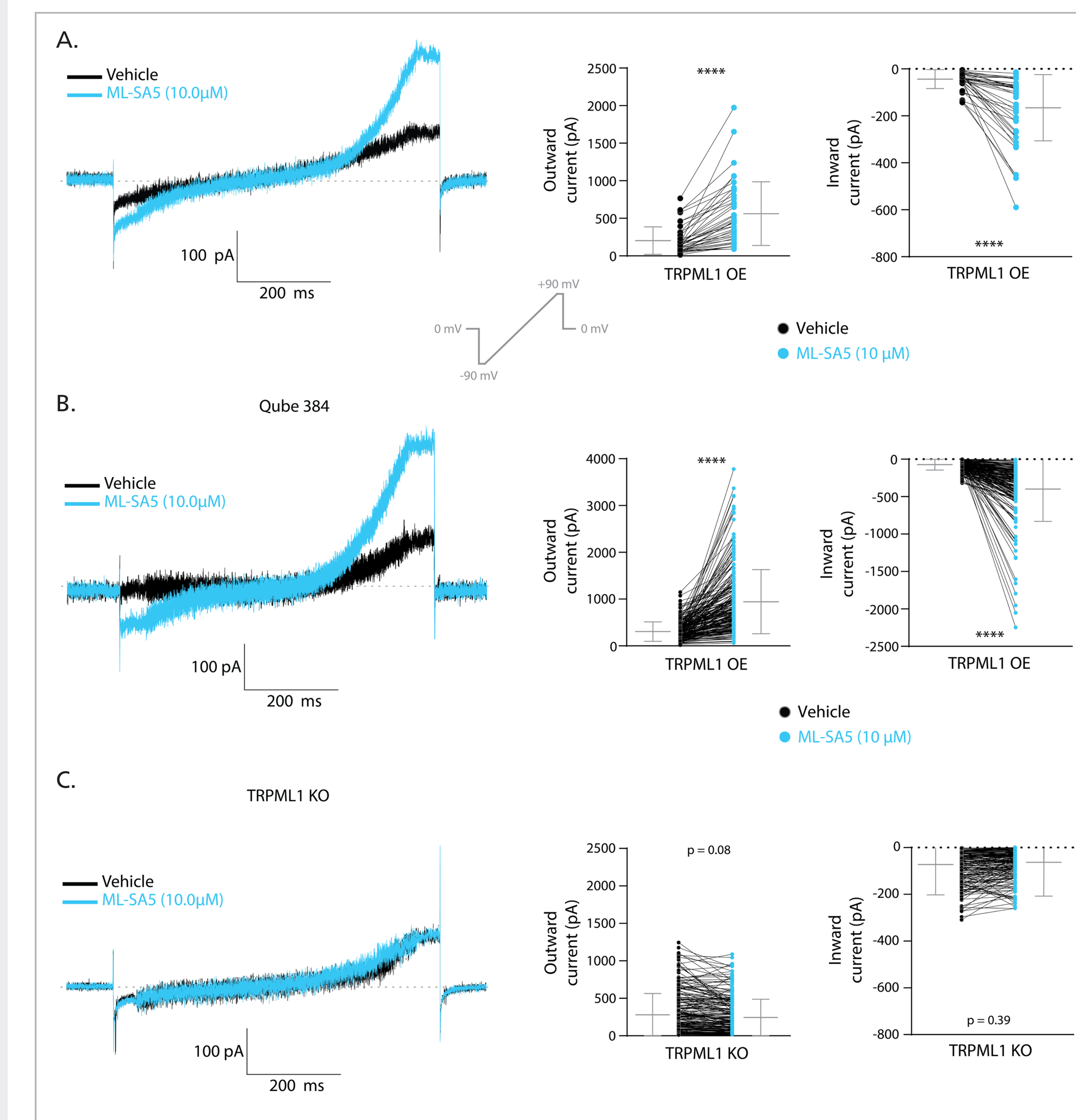

Fig. 4: Pharmacological activation of TRPML1 current in overexpressing (OE) lysosomes. (A) Representative current traces recorded on QPatch 48 before (black) and after (blue) application of 10 µM ML-SA5. Both inward and outward currents were enhanced upon compound application. (B) Corresponding recordings from Qube 384 showing a similar increase in TRPML1-mediated currents following 10 µM ML-SA5. Out of the successfully captured lysosomes, 41.3 ± 1.9% (n = 4 plates) expressed an ML-SA5 sensitive current on the QPatch 48 and 50.3 ± 5.0% on the Qube 384 (n = 4 plates). (C) Recordings from TRPML1-knockout (KO) lysosomes under identical conditions. No increase in inward or outward current was observed after ML-SA5 application, confirming TRPML1 specificity. Currents were measured at +90 (outward) and -90 mV (inward), 600 ms voltage ramp is shown in grey in panel A. Grey bars in graphs represent mean ± SD. **** = $p < 0.0001$, OE = overexpressed, KO = knockout.

Table 3: Current values in single concentration activation experiments. Values are represented as mean ± SEM. KO = knockout

Current (pA)	QPatch (n = 43)	Qube (n = 208)	TRPML1 KO (n = 221)
Outward current vehicle	203 ± 29	307 ± 14	289 ± 20
Outward current ML-SA5	562 ± 67	946 ± 47	252 ± 17
Inward current vehicle	-43 ± 6	-76 ± 5	-72 ± 9
Inward current ML-SA5	-165 ± 21	-403 ± 30	-62 ± 10

Concentration response to TRPML1-specific agonist ML-SA5

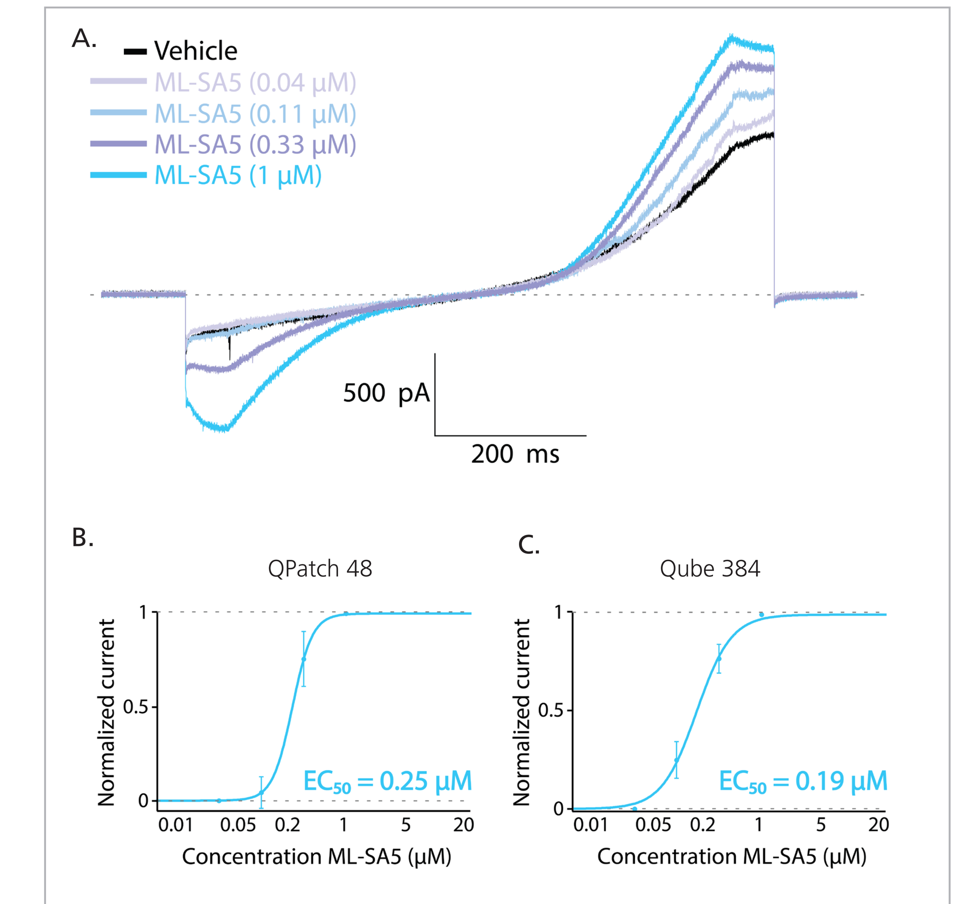

Fig. 5: ML-SA5 Concentration Response Curve on QPatch 48 and Qube 384. (A) Representative current traces from a cumulative concentration response experiment on QPatch 48 with increasing concentrations of ML-SA5 from 37 nM to 1 µM. (B) Average concentration-response curve for ML-SA5 activation of TRPML1 on QPatch 48 (EC50 = 0.25 µM, nHill = 3.87). (C) Average concentration-response curve for ML-SA5 activation of TRPML1 on Qube 384 (EC_{50} = 0.19 µM, nHill = 2.22). These potencies and hill coefficients are consistent with literature values. Currents were measured at +90 mV, voltage ramp is shown in Figure 3. Data shown represents the mean ± SEM.

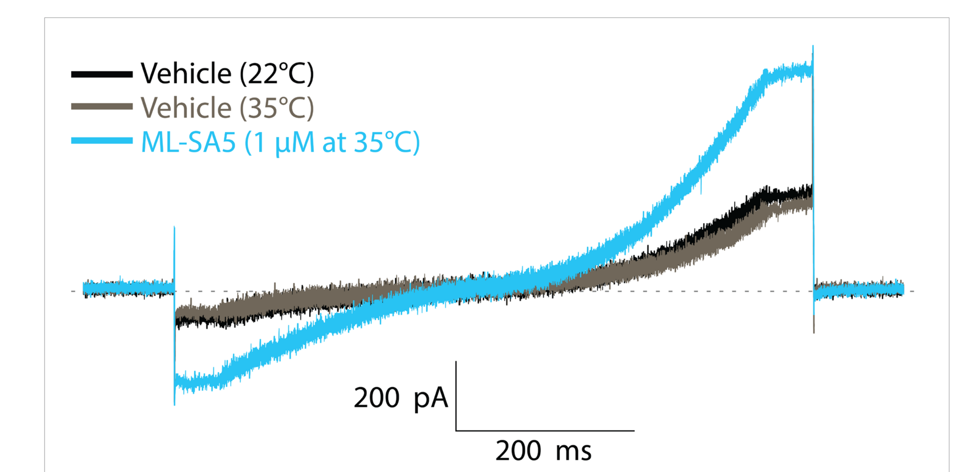

Fig. 6: TRPML1 activation at physiological temperature. Representative current traces recorded on Qube 384 from one site during the same experiment at 22° C (black), 35° C (grey) and with ML-SA5 application at 35°C (blue). The success rate was 71.1%. Voltage ramp is shown in Figure 3.

Materials and methods

Patch clamp experiment: All patch clamp experiments were carried out using the QPatch 48 or Qube 384 platform (Sophion Bioscience A/S, Denmark).

Lysosomes: LYSO-Prep™ were obtained from Oria Bioscience (Paris, France).

Experimental setup: Experiments were conducted using single-hole high-resistance consumables (HiR) for either the QPatch 48 or Qube 384 platforms. LYSO-Prep™ from Oria Bioscience were stored at -80° C for up to four weeks and thawed on the day of experiments for immediate use. For TRPML1, Na^+ containing solutions were used.

Whole-cell protocol: Using Qube 384 differential whole-cell plate capabilities a lysosome specific protocol was optimized to gently break into fragile membranes without bursting.

Voltage protocol: Lysosomes were held at 0 mV throughout the experiment. Currents were evoked using a 600 ms ramp from -90 mV to +90 mV.

QC filters: For determining successful lysosome capture, we used membrane resistance > 200 MΩ.

Conclusion

Automated Patch Clamp of lysosomal ion channels was achieved using enlarged lysosomes (LYSO-Prep™, Oria Bioscience), enabling measurement of ion channel activity such as TRPML1. Lysosomes were successfully captured on two Sophion systems, with success rates of 69.9 ± 3.2% on the QPatch 48 and 74.6 ± 2.6% on the Qube 384. The lysosomal recordings showed stable seals and robust TRPML1 current responses. Agonist concentration–response curves produced EC_{50} values that were reproducible on both platforms and in good agreement with published data[8]. Based on these results, Sophion's medium- and high-throughput APC systems provide a reliable and scalable approach for studying lysosomal ion channel pharmacology, including TRPML1.

Presented @BPS2026