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Application Report

Breaking barriers in lysosomal electrophysiology: Automated solutions for ion channel analysis

From reliable lysosome capture to robust TRPML1 pharmacology on Sophion's platforms

Summary

Automated Patch Clamp (APC) 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.

Introduction

Lysosomes are essential cellular organelles responsible for degrading and recycling macromolecules, maintaining cellular homeostasis, and regulating processes such as autophagy and membrane trafficking[1]. In recent years, lysosomal ion channels have emerged as critical regulators of these functions, with particular relevance to neurodegenerative diseases. Dysregulation of lysosomal activity and impaired clearance of protein aggregates have been implicated in disorders such as Parkinson's disease, Alzheimer's disease, and Amyotrophic Lateral Sclerosis (ALS), highlighting the therapeutic potential of targeting lysosomal pathways[2-4].

Among lysosomal ion channels, TRPML1 is a well-characterized member of the transient receptor potential family, mediating Ca^{2+} release from lysosomes[5]. Its activity is crucial for lysosomal trafficking, membrane repair, and autophagosome-lysosome fusion[6,7]. Proper assessment of TRPML1 function provides insights into lysosomal physiology and disease mechanisms, making it an important target for pharmacological research.

Studying lysosomal ion channels, however, presents unique challenges. Lysosomes are small, dynamic organelles, and conventional patch clamp approaches are labor-intensive and technically demanding. Sophion Bioscience has developed a high-throughput automated patch clamp method for enlarged lysosomes (LYSO-Prep™, Oria Bioscience), which overcomes these limitations. Using small-hole, high-resistance consumables, the APC platforms - QPatch 48 and Qube 384 - enable reliable capture of individual lysosomes and formation of stable seals suitable for electrophysiological recordings. This approach allows precise measurement of lysosomal ion channel currents, including TRPML1, and supports reproducible pharmacological studies, including the generation of concentration–response data. This assay supports scalable studies of lysosomal ion channel function, disease mechanisms, and the evaluation of potential therapeutic strategies.

Results and discussion

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 (n = 10 plates) and 74.6 ± 2.6% on the Qube (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).

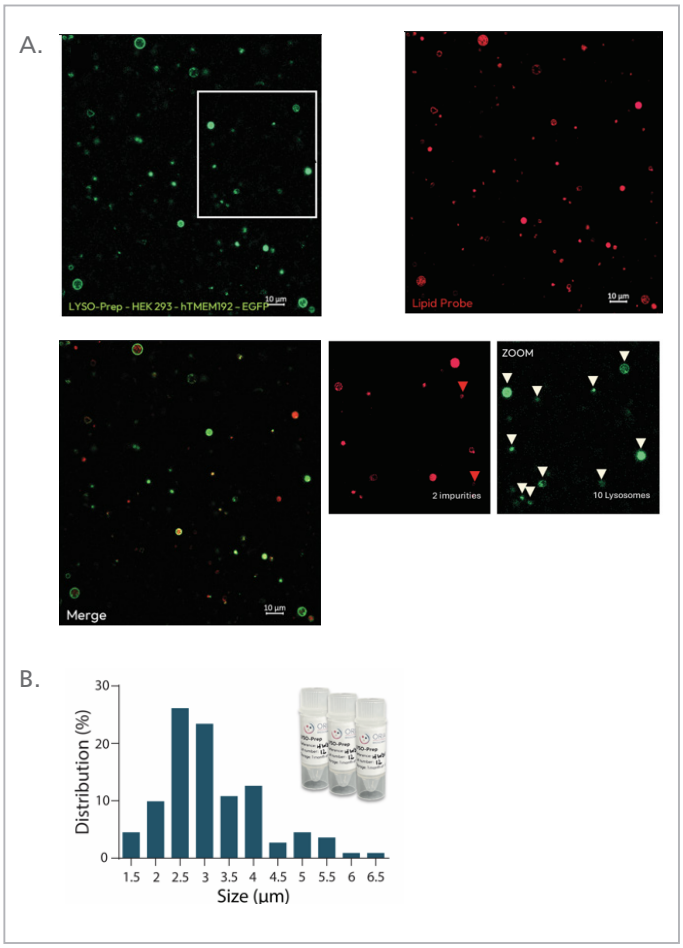

Fig. 1: LYSO-Prep™ from Oria Bioscience. (A) Confocal microscopy images of LYSO-Prep™ from HEK293 cells overexpressing hTMEM192-EGFP and labeled with a lipid probe (staining all membranes), illustrating the purity of the samples (63× oil objective). **(B)** Standard size distribution of LYSO-Prep™ following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

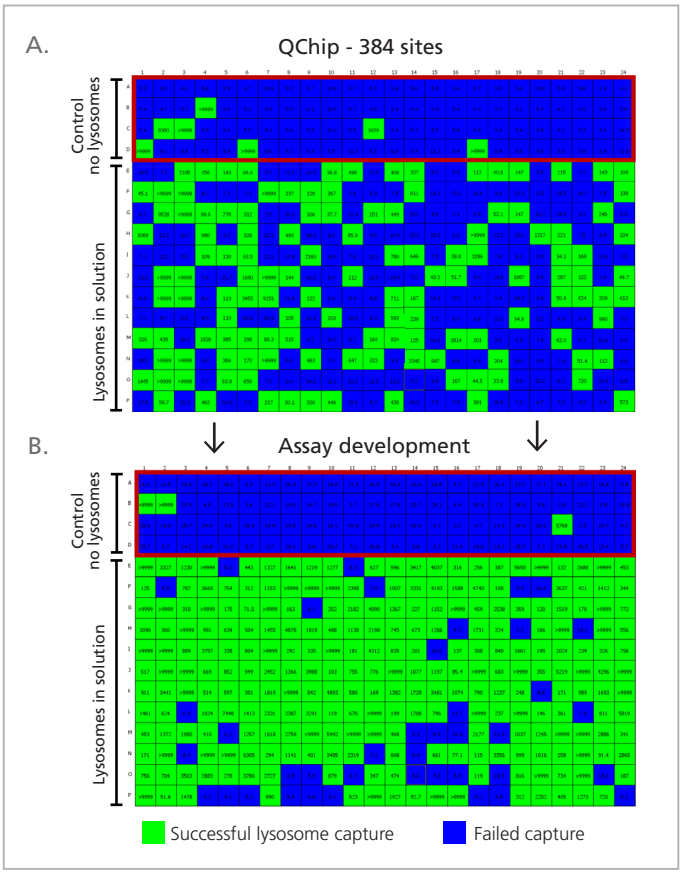

Fig. 2: Assay development increased successful lysosome capture rates. Two example plate images from Qube illustrating the distribution of successful lysosome captures. The first 4 rows (A to D) were used as negative controls (no lysosomes in solution). **Top**: One of the first experiments with low 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 Qube (n = 10 plates) and 69.9 ± 3.2% on QPatch (n = 10 plates; plate images not shown). QC filters were applied (see Methods section).

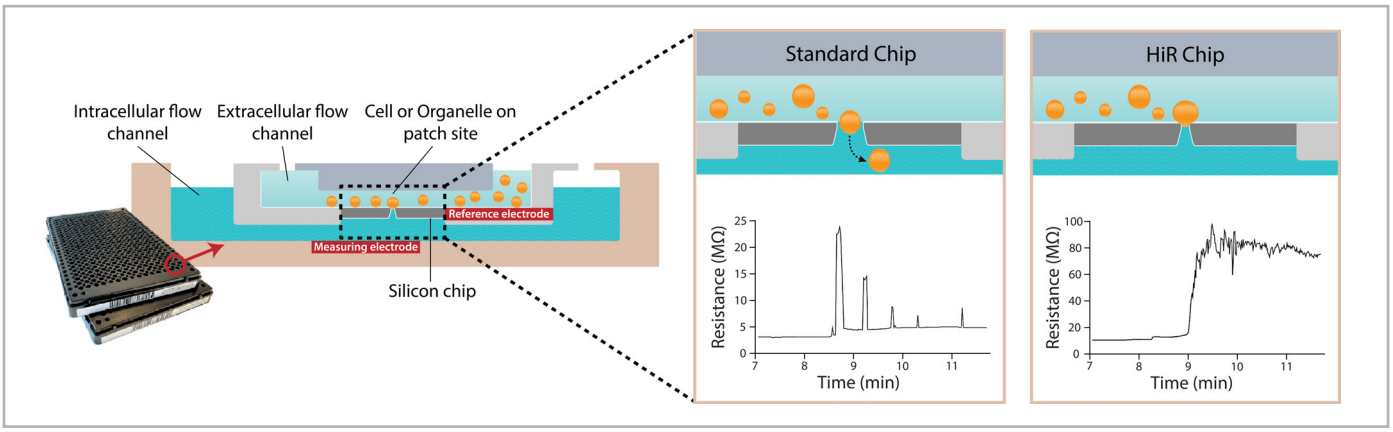

Fig. 3: **High-resistance consumables reduce lysosome slipping.** A cross-section illustration of a small-hole, high-resistance (HiR) consumable showing the reduction of lysosome slipping and successful lysosome capture. On a standard chip lysosome slipping can be observed on the resistance plot during lysosome capture, on a HiR chip this slipping is reduced, and lysosome capture can be confirmed by rapid and persistent increase in resistance.

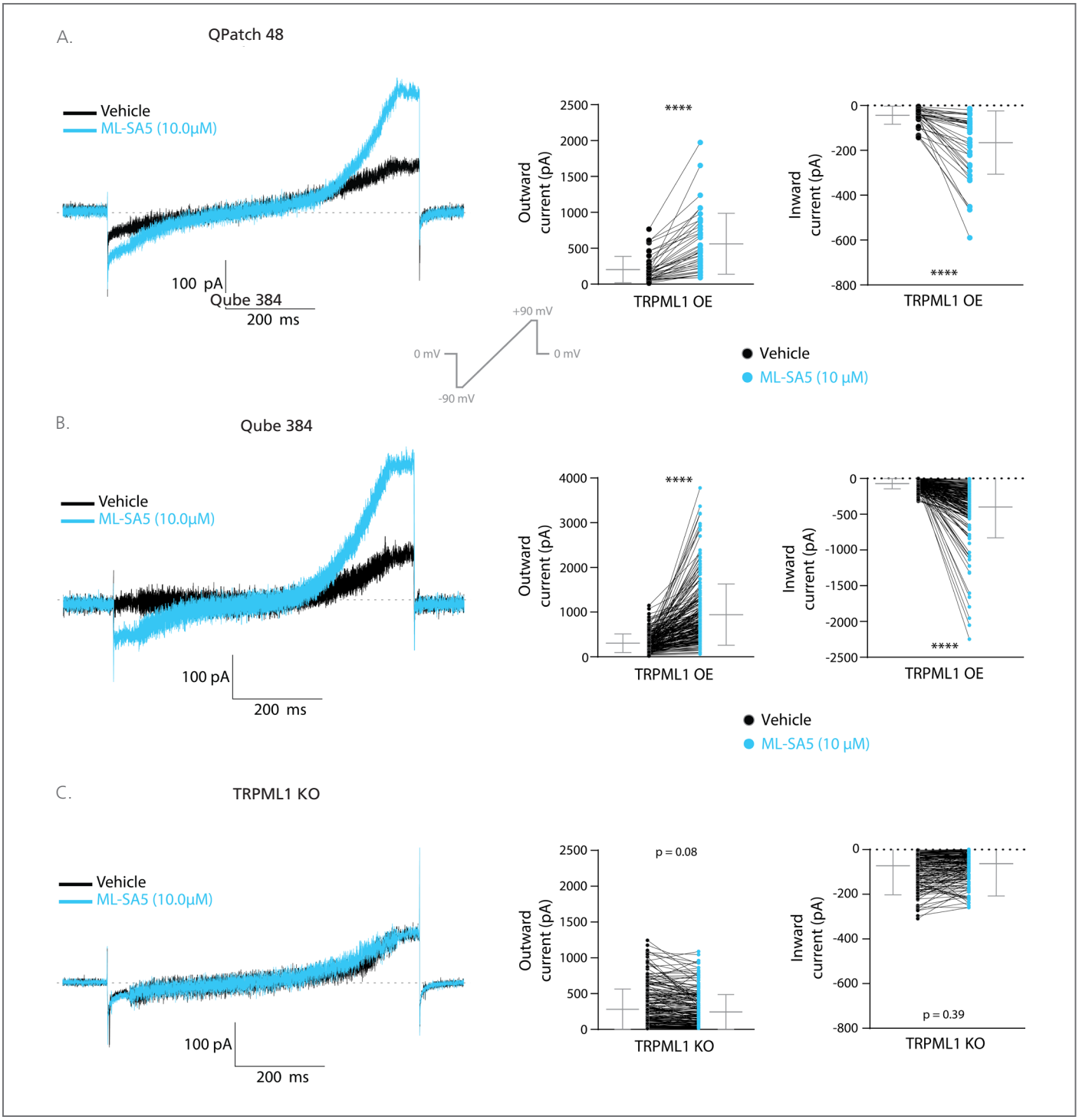

Figure. 4: Pharmacological activation of TRPML1 current in overexpressing (OE) lysosomes. (A) Representative current traces recorded on QPatch before (black) and after (blue) application of 10 µM ML-SA5. Both inward and outward currents were enhanced upon compound application. **(B)** Corresponding recordings from Qube 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 QPatch, 50.3 ± 5.0% on Qube (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), voltage ramp is shown in grey in panel A. Grey bars in graphs represent mean ± SD. **** = $p < 0.0001$, OE = overexpressed, KO = knockout.

Pharmacological validation of TRPML1 current

Next, we assessed channel activity in lysosomes overexpressing TRPML1 using the selective agonist ML-SA5. Application of single 10 µM concentration of ML-SA5 activated the TRPML1 channel and significantly enhanced the outward and inward current on both QPatch and Qube (Figure 4, Table 1). Out of the successfully captured lysosomes, 41.3 ± 1.9% (n = 4 plates) expressed an ML-SA5 sensitive current on QPatch, 50.3 ± 5.0% on Qube (n = 4 plates).

As an additional control we performed a single-concentration experiment using TRPML1-knockout lysosomes. The application of 10 µM ML-SA5 did not elicit any increase in current (Figure 4C) indicating both that the compound is a specific activator of this channel, but also that what we measure in our previous experiments is indeed TRPML1 current.

Table 1: 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

To further quantify activation, we constructed concentration–response curves by applying increasing ML SA5 concentrations (37 nM – 1 µM). Analysis of the outward current measured at +90 mV resulted in EC_{50} values of 0.25 µM (n_{Hill} = 3.87) on QPatch and 0.19 µM (n_{Hill} = 2.22) on Qube. These potencies and hill coefficients are consistent with literature values for ML SA5 activating TRPML1 in whole-endolysosomal patch clamp[8].

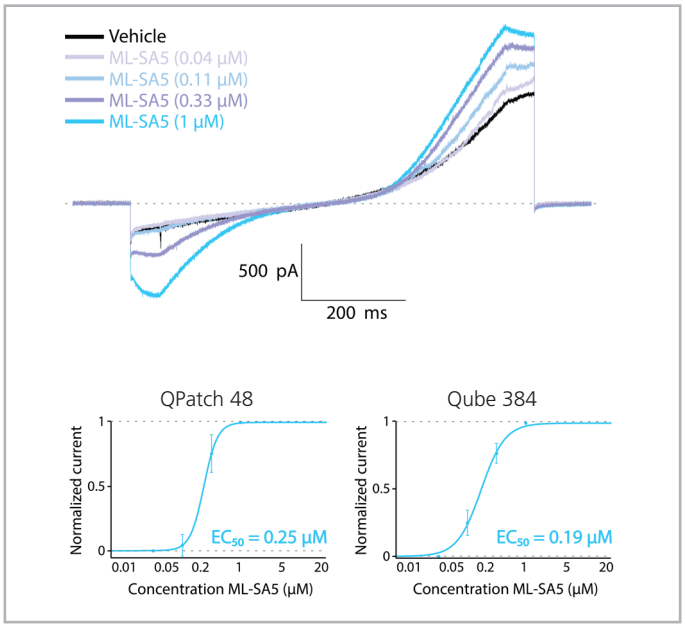

Figure 5: ML-SA5 Concentration Response Curve on QPatch and Qube. (A) Representative current traces from a cumulative concentration response experiment on QPatch with increasing concentrations of ML-SA5 from 37 nM to 1 µM. **(B)** Average concentration-response curve for ML-SA5 activation of TRPML1 on QPatch (EC_{50} = 0.25 µM, n_{Hill} = 3.87). **(C)** Average concentration-response curve for ML-SA5 activation of TRPML1 on Qube (EC_{50} = 0.19 µM, n_{Hill} = 2.22). These potencies and hill coefficients are consistent with literature values8. Currents were measured at +90 mV, voltage ramp is shown in Figure 3. Data shown represents the mean ± SEM.

Lysosomal recordings at physiological temperature

We then performed a concentration experiment at 35° C on Qube. The success rate was 71.1% (n = 1 plate), comparable to experiments at 22° C (74.6 ± 2.6%). Therefore, it is possible to do high throughput pharmacological assessment of TRPML1 using LYSO-Prep™ at physiological temperatures without a drop in success rates.

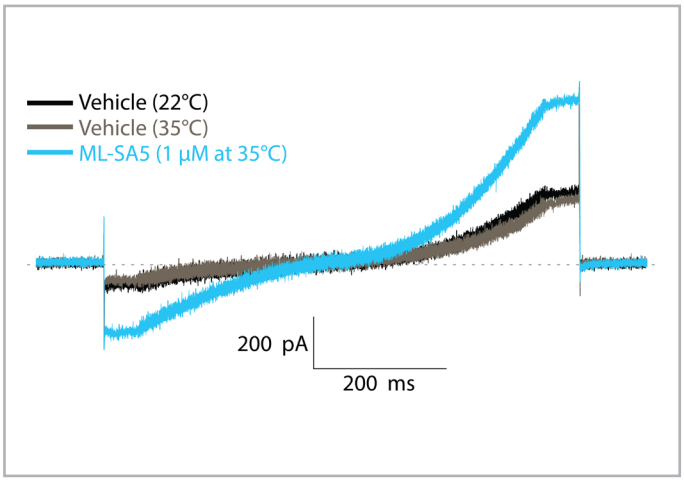

Figure 6: TRPML1 activation at physiological temperature. Representative current traces recorded on Qube from one site during the same experiment at 22° C (black), 35° C (grey) and with ML-SA5 application at 35° C (blue). The success rate was 71.1%. Voltage ramp is shown in Figure 3.

Methods

- **Patch clamp experiment**: All patch clamp experiments were carried out using the QPatch 48 or Qube 384 platforms (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.
- **Whole-cell protocol**: Using the Qube 348 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

For information on sample preparations and solutions contact us at info@sophion.com.

References

1. Saftig P, Klumperman J. Lysosome biogenesis and lysosomal membrane proteins: trafficking meets function. Nature Reviews Molecular Cell Biology. 2009 Aug 12;10(9):623–35.
2. Nixon RA. The role of autophagy in neurodegenerative disease. Nature Medicine. 2013 Aug;19(8):983–97.
3. Udayar V, Chen Y, Sidransky E, Jagasia R. Lysosomal dysfunction in neurodegeneration: emerging concepts and methods. Trends in Neurosciences. 2022 Mar;45(3):184–99.
4. Kett LR, Dauer WT. Endolysosomal dysfunction in Parkinson's disease: Recent developments and future challenges. Movement Disorders. 2016 Oct;31(10):1433–43.
5. Xu H, Ren D. Lysosomal Physiology. Annual Review of Physiology. 2015 Feb 10;77(1):57–80.
6. Dong XP, Cheng X, Mills E, Delling M, Wang F, Kurz T, et al. The type IV mucolipidosis-associated protein TRPML1 is an endolysosomal iron release channel. Nature. 2008 Sep 14;455(7215):992–6.
7. Cao Q, Yang Y, Zhong XZ, Dong XP. The lysosomal Ca^{2+} release channel TRPML1 regulates lysosome size by activating calmodulin. Journal of Biological Chemistry. 2017 May 1;292(20):8424–35.
8. Yu L, Zhang X, Yang Y, Li D, Tang K, Zhao Z, et al. Small-molecule activation of lysosomal TRP channels ameliorates Duchenne muscular dystrophy in mouse models. Science Advances. 2020 Feb 1;6(6):eaaz2736.

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