Application Note

A Multi-Platform Approach to TMEM175 Drug Discovery

Introduction

TMEM175 is a novel, constitutively active ion channel involved in the regulation of lysosomal pH, lysosomal Δψ and autophagy. TMEM175's significance lies not only in its physiological role but also in its therapeutic promise. Dysfunction of TMEM175 has been associated with lysosomal storage disorders and neurodegenerative diseases such as Parkinson's and Alzheimer's. As such, understanding TMEM175's role in health and disease may provide opportunities for therapeutic interventions targeting these conditions.
SB Drug Discovery stands at the forefront of TMEM175 research, offering an array of specialized services and cutting-edge capabilities to understand this target's therapeutic potential.

Supporting Your Research

A lack of specific pharmacological tools has hampered detailed characterization of the role of TMEM175 in normal lysosomal function and related pathological processes. SB Drug Discovery has developed an extensive catalogue of ion channel cell lines including a spectrum of TMEM175 reagents such as wild type and Loss/Gain of Function (LoF/GoF) variants, alongside a variety of species orthologs.

TMEM175 cell lines		
TMEM175 (human)	TMEM175 (loss/gain of function)	TMEM175 (mouse)
TMEM175 (rat)	TMEM175 (dog)	TMEM175 (monkey)

Our comprehensive suite of TMEM175 assays and technologies is tailored to address diverse research objectives with precision and efficiency. For high-throughput screening needs, 384-well fluorescence-based and electrophysiology platforms enabling rapid hit identification. Complimenting this, automated electrophysiology platforms support multiple assay formats, measuring both potassium conductance and proton-activated proton conductance at low pH. Together with novel, cutting-edge solid supported membrane based electrophysiology our comprehensive capabilities help support TMEM175 drug discovery from hit identification to lead optimization and beyond.

TMEM175 Discovery Platform

- **Off-the-Shelf Reagents**
  Wild-type, disease-relevant variants and species orthologs.
- **Hit Identification**
  Fluorescence-based high-throughput screening and downstream assay cascades.
- **Cutting-Edge Technologies**
  High-throughput automated electrophysiology platforms for HTS and lead-optimization.

Orthogonal assays using SSM-electrophysiology enabling lysosomal channel studies.

Methods

- Automated whole-cell patch-clamp experiments were carried out using the SyncroPatch 384i platform.
- TMEM175 currents were monitored using a voltage protocol consisting of a 500 ms ramp from -80 mV to +80 mV, followed by a 500 ms step from -80 mV to +80 mV before returning to the holding potential of 0 mV. Voltage sweeps were repeated every 10 seconds and currents were sampled at 10 kHz. The maximum inward current amplitude obtained at -80 mV and the maximum outward current amplitude obtained at +80 mV from the ramp section were used for analysis.
- Solid Supported Membrane (SSM) Electrophysiology measurements were carried out using the SURFE^{2}R 96SE (Nanion Technologies), using 96-well, gold coated sensor chips.
- A fast solution exchange from non-activating solution (NA) to activating solution (A) at 0 mV, applied a substrate gradient to activate charge translocation through TMEM175, immobilized on a gold-coated sensor chip.

Assay Validation

SB Drug Discovery employs a comprehensive approach to validating and characterizing its recombinant ion channel cell lines. Our assay validation framework involves meticulous examination of functionality, including pharmacological profile, assay stability and reproducibility. These TMEM175 assays have been extensively optimized to generate robust, reproducible responses and deliver high-quality electrophysiology data suitable for high-throughput screening as well as the demands of downstream lead optimization.

Figure 1. A representative schematic of the TMEM175 electrophysiology application protocol.

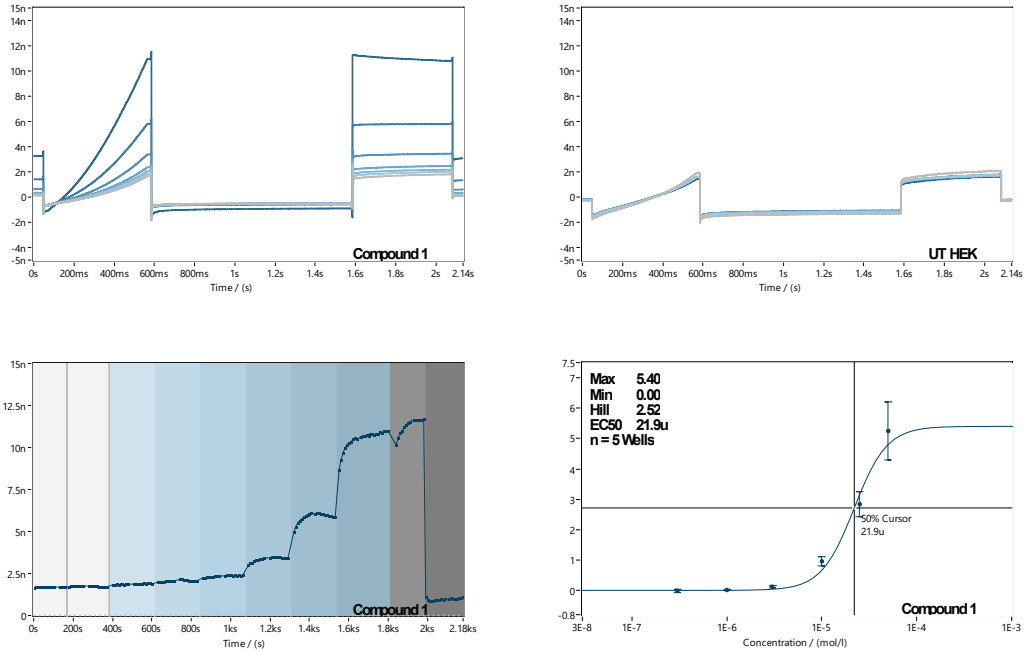

Figure 2. Representative current traces, time course and concentration response curve showing the effect of the reference activator against TMEM175 channels and untransfected cells.
The application protocol consists of addition of extracellular physiological solution, followed by increasing concentrations of test compound. A max concentration of reference activator is then applied, before application of saturating concentration of inhibitor.

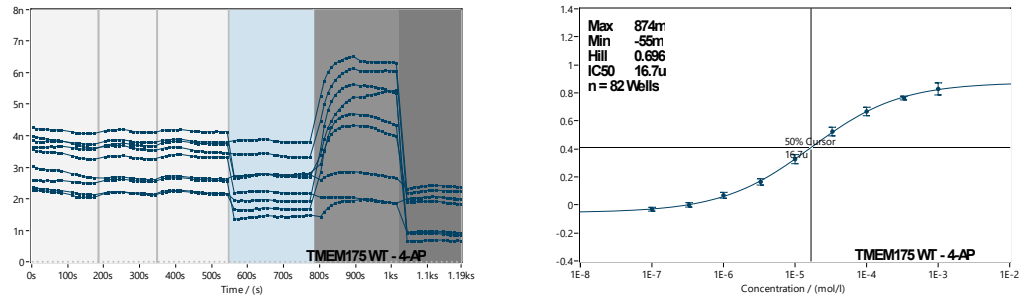

Figure 3. Representative time course and concentration response curve showing the effect of inhibitor 4-AP against TMEM175.
The application protocol consists of addition of extracellular physiological solutions, followed by application of increasing concentrations of 4-AP. Reference activator is then applied before the channel is inhibited by a saturating concentration of 4-AP.

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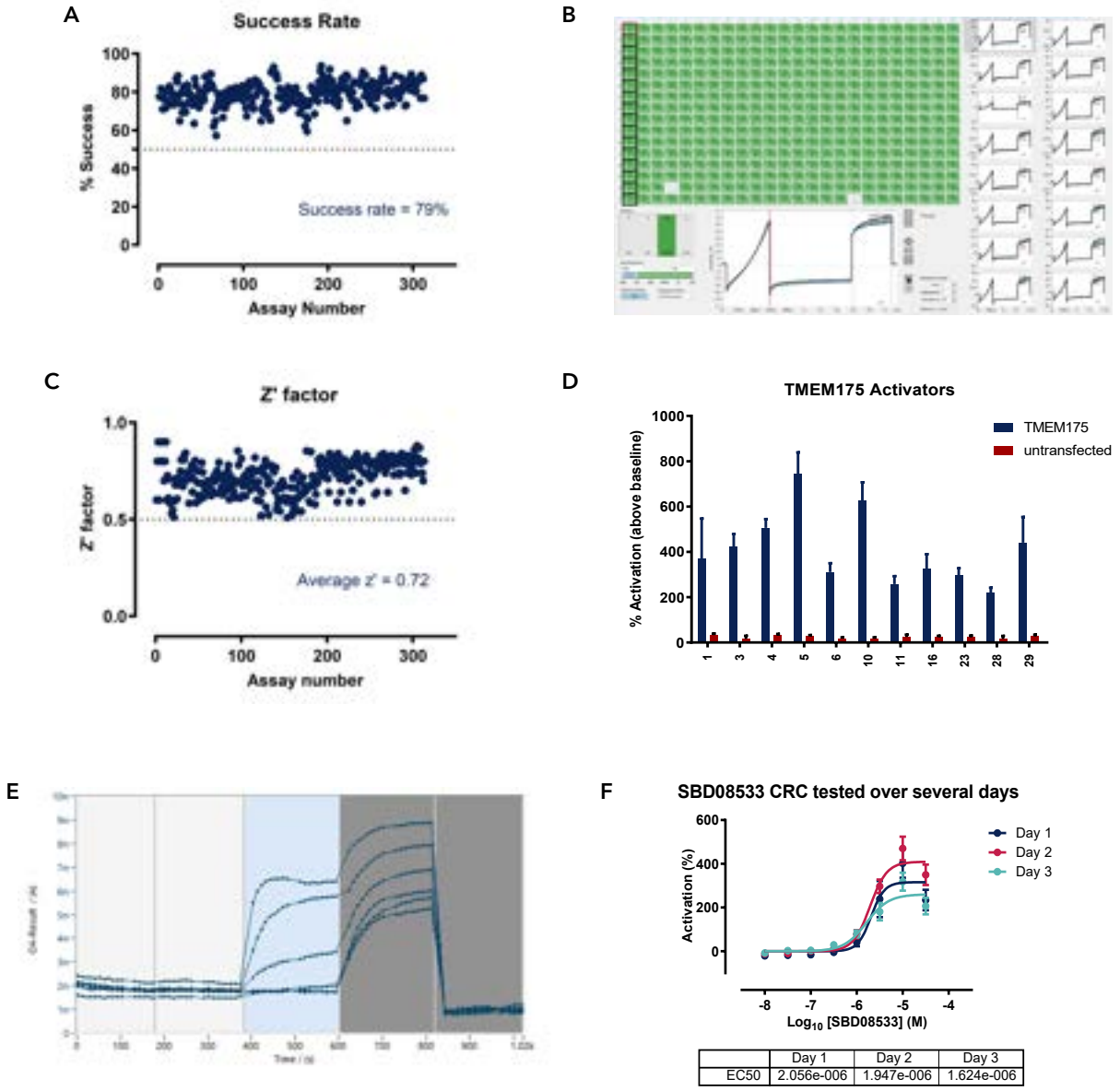
Hit Identification

Utilizing state-of-the-art 384-well automated electrophysiology platforms, SB Drug Discovery has successfully conducted numerous TMEM175 screening campaigns, interrogating >30,000 small molecules at a single concentration. This screening assay has successfully identified many active compounds showing the ability to modulate TMEM175 in a concentration-dependent manner. The successful development of a TMEM175 electrophysiology assay, equipped to identify novel pharmacological tools, now facilitates an in-depth investigation into the role of TMEM175 in both normal physiology and disease states.



	Day 1	Day 2	Day 3
EC50	2.056e-006	1.947e-006	1.624e-006

Figure 4. Discovery of novel TMEM175 modulators via high-throughput electrophysiological screening.
(A & C) Average success rate and Z' factor of TMEM175 HTS assay. Screening assay yielded an average success rate of 79% (wells passing QC) and an average Z' factor of 0.72. (B) Screenshot of DataControl384 used for automated analysis of 384 parallel recordings. (D) Hit confirmation showing % activation against TMEM175 and untransfected cells. (E) Representative time course showing TMEM175 activator against the TMEM175. (F) Normalised concentration response curve showing reproducible activation by example hit compound (SBD08533) tested over several days.

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Proton Conductance

SB Drug Discovery has validated a high throughput screening assay in which pH is used to activate TMEM175. By establishing a proton gradient across the cell membrane, a pH-dependent inward proton current can be measured which increases in relation to acidification. This proton conductance can be modulated by known TMEM175 activators and blocked by the reference inhibitor 4-AP. TMEM175 activators, identified via HTS, were also shown to modulate TMEM175 in a concentration-dependent manner. The low-pH TMEM175 assay described here complements existing TMEM175 screening tools and demonstrates the potential to identify and characterize novel pharmacological tools for TMEM175 drug discovery.

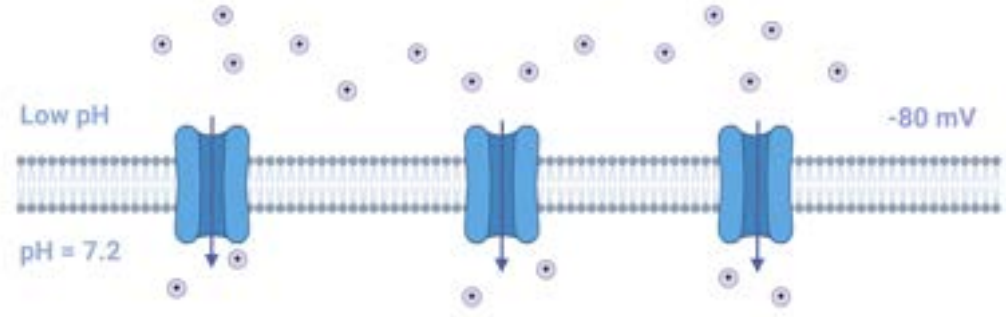

Figure 5. **Proton conductance assay illustrating activation of TMEM175 by lysosomal physiological conditions.**

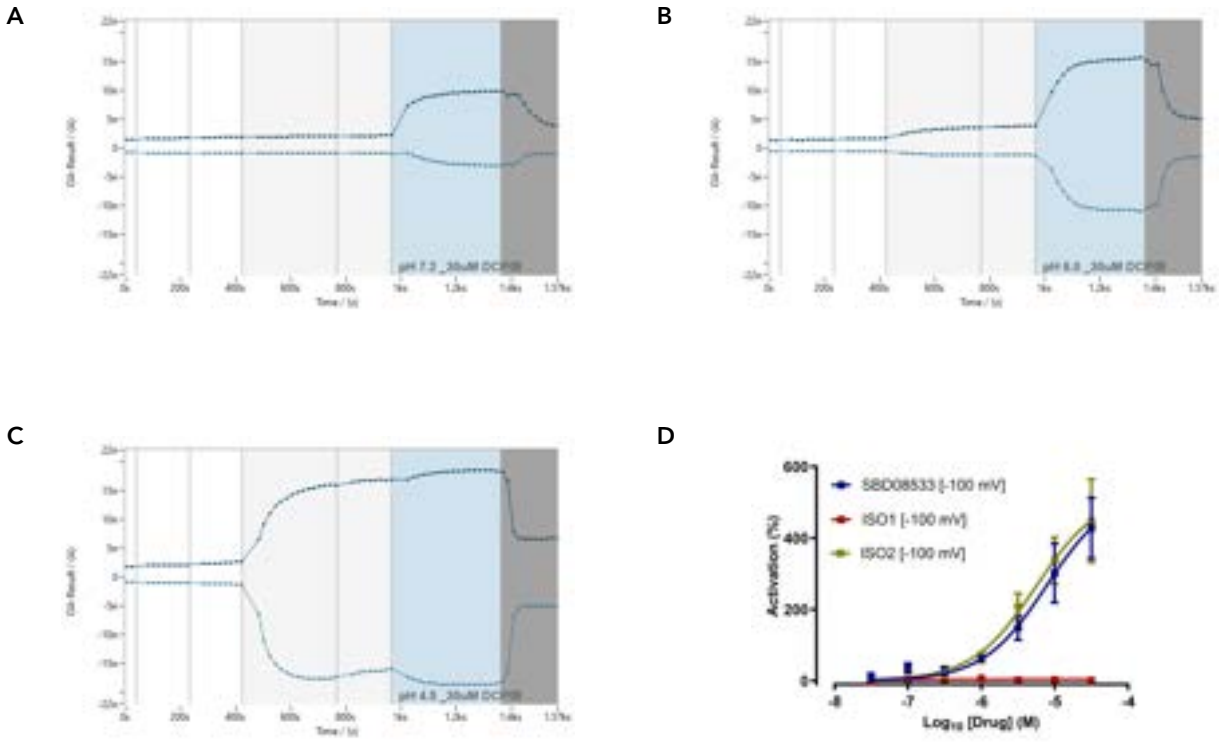

Figure 6. **Activation of TMEM175 proton conductance at pH 4.5, 6.0 and 7.2.**
Representative time course plots showing the effect of reference TMEM175 activator DCPIB on TMEM175 at pH 7.2, 6.0 and 4.5 (A,B,C). The application protocol consists of extracellular physiological solution at pH 7.2, followed by an application of desired pH. DCPIB is then applied before channels are inhibited by a saturating concentration of reference inhibitor. (D) Concentration-dependent effect of hit compound SB08533 and its enantiomers ISO-1 and ISO-2 at pH 6.0.

Disease Relevant Variants

A number of TMEM175 variants have been associated with neurodegenerative disorders such as Parkinson's disease. Our gain of function and loss of function TMEM175 assays enable functional characterization disease relevant variants and provide insight into the modulatory effect of TMEM175 targeting molecules.

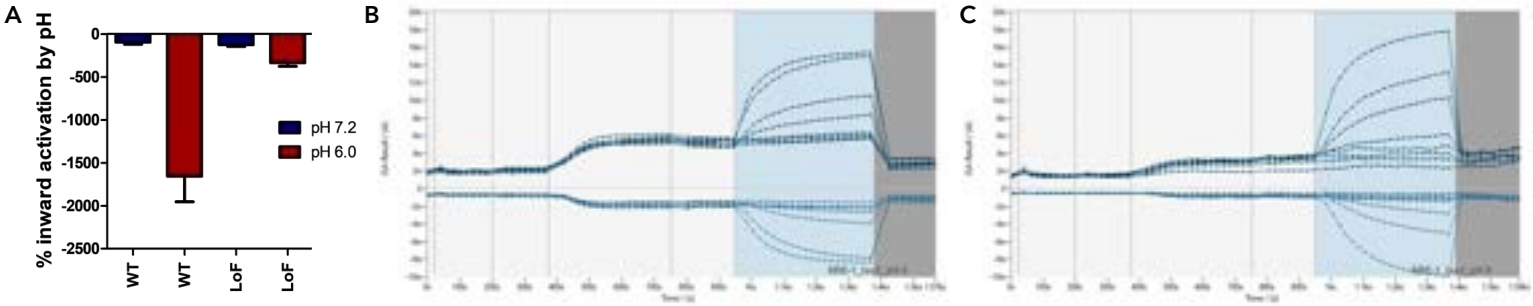

Figure 7. Effect of pH 7.2 and pH 6.0 on Wild Type (WT) and Loss of Function (LoF) TMEM175 cells.
(A) % inward activation induced by pH 7.2 and pH 6.0 on WT and LoF overexpressing TMEM175 cells. (B & C) Representative time course plots showing the degree of activation induced by standard pH 6.0 for WT (B) and LoF (C) TMEM175 cells.

Lysosomal Studies

Solid supported membrane (SSM)- based electrophysiology enables the characterization of ion fluxes through TMEM175 expressed in lysosomal membranes. This technology provides an orthologous method to assess TMEM175 modulators identified via high-throughput screening and conventional electrophysiology assays. SSM electrophysiology, performed using 96-well SURFE^{2}R technology, can be used to assess both activators and inhibitors of TMEM175 in a high-throughput format.

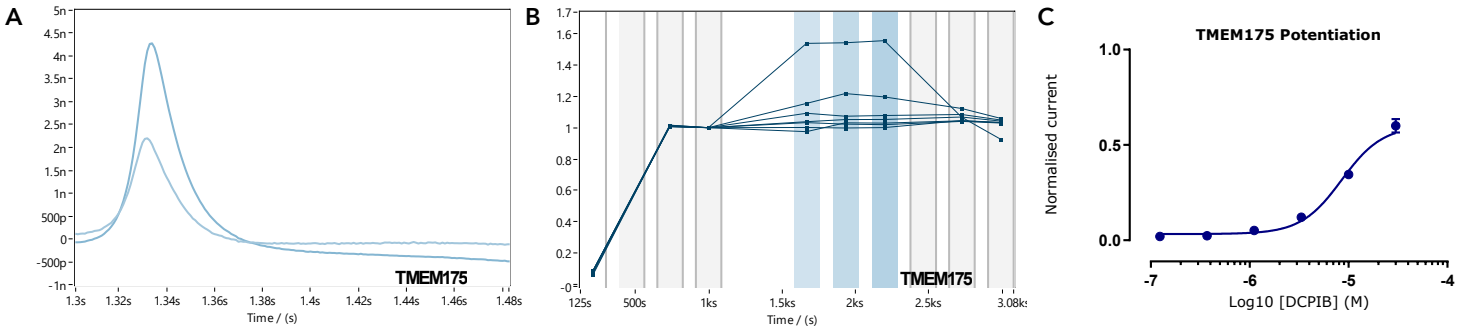

Figure 8. Activation of lysosomal TMEM175.
A) Activation of lysosomal TMEM175 by KCl and potentiation by DCPIB. B) Time course recording showing potentiation of K^+ conductance by DCPIB. Following control measurement (baseline), solution exchange from 20 mM NaCl to 20 mM KCl enables measurement of K^+ flux via TMEM175. Sensors are then washed and solution exchange repeated in the presence of test compound (blue columns). (C) Concentration response curve showing the effect of DCPIB on lysosomal TMEM175.

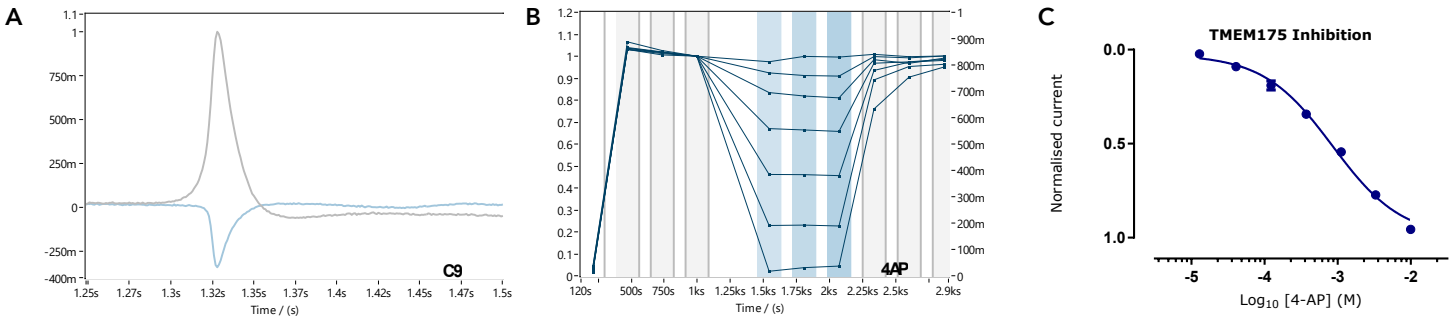

Figure 9. Inhibition of lysosomal TMEM175
A) Activation of lysosomal TMEM175 by KCl and inhibition by 4-AP. B) Time course recording showing inhibition of K+ conductance by 4-AP. Following control measurement (baseline), solution exchange from 50 mM NaCl to 50 mM KCl enables measurement of K^+ flux via TMEM175. Sensors are then washed and solution exchange repeated in the presence of test compound (blue columns). (C) Concentration response curve showing the effect of 4-AP on lysosomal TMEM175.

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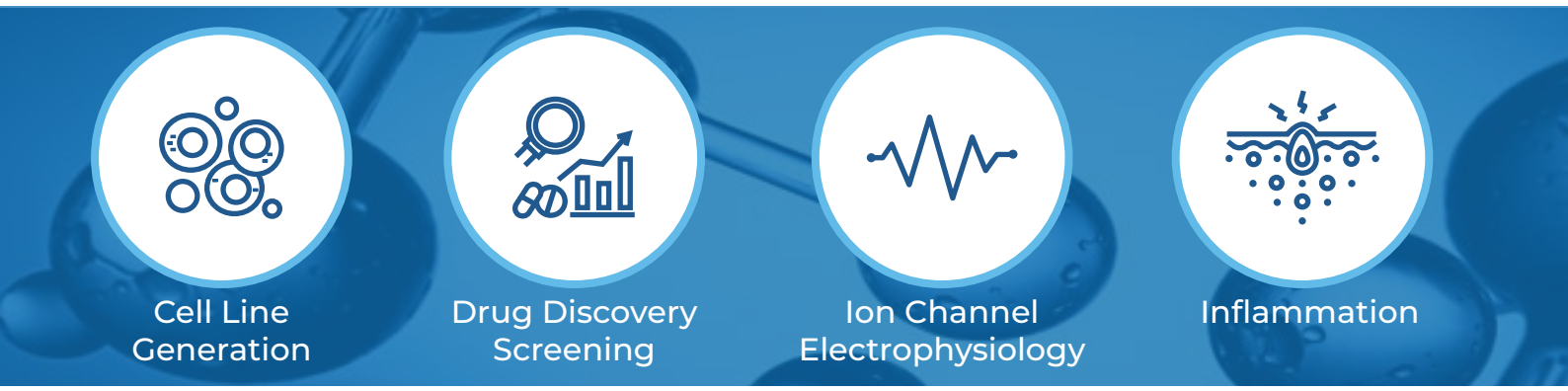
SB Drug Discovery's Commitment to Ion Channel Research

SB Drug Discovery is proud to play a role in the search for new therapeutics with our extensive suite of TMEM175 drug discovery tools and decades of expertise. Our advanced automated patch clamp, high throughput screening, and lead optimization capabilities position SB Drug Discovery as the premier provider for TMEM175 drug discovery research.

Our discovery platform provides a wide array of resources for TMEM175 research, including readily available reagents encompassing wild-type, disease-relevant variants, and species orthologs. Leveraging fluorescence-based high-throughput screening and downstream assay cascades, our hit identification services quickly identify promising small molecule modulators. We employ cutting-edge technologies such as high-throughput automated electrophysiology enabling streamlined screening and lead optimization cascades. Additionally, our orthogonal assays employing solid-supported membrane-based electrophysiology allow for intricate investigations of lysosomal channels, ensuring comprehensive support for TMEM175 drug discovery from initial hit identification through lead optimization and beyond.



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