Identification of novel TMEM175 modulators using high-throughput automated patch-clamp and solid-supported membrane- (SSM-) based electrophysiology platforms

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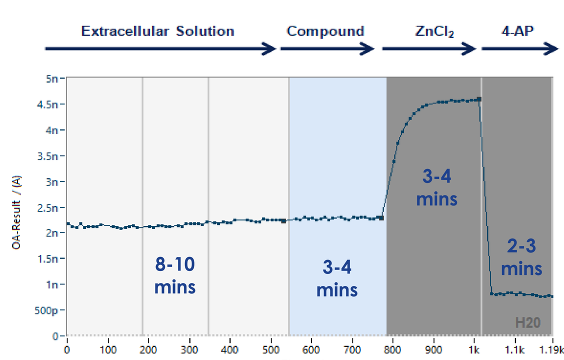
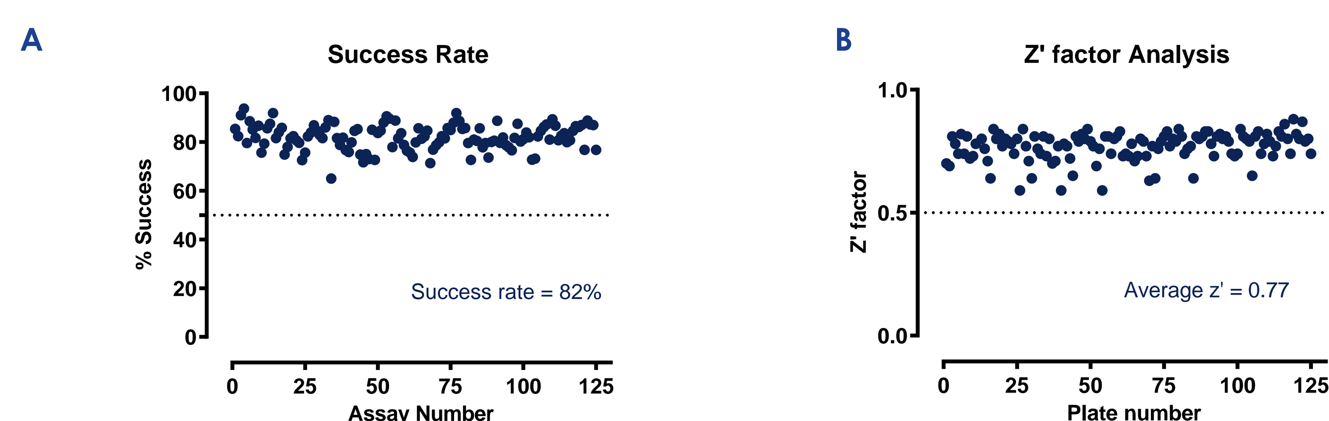
1 Introduction

TMEM175 is a novel, constitutively active ion channel involved in regulating lysosomal pH and autophagy. Mutations in this gene impair normal lysosomal and mitochondrial function, thereby increasing aggregation of insoluble proteins such as phosphorylated a-synuclein, leading to symptoms typical of Parkinson's Disease (PD). Consequently, TMEM175 demonstrates significant potential as a key player in the treatment of PD. The lack of specific pharmacological tools has hampered further investigation into the exact role of TMEM175 in normal lysosomal function and pathological processes. Here, the TMEM175 stable cell line was characterized using automated patch-clamp and SSM-based electrophysiology. We developed and executed robust, high-throughput, and high-content direct electrophysiological intracellular screening assays, with exceptionally high success rates.

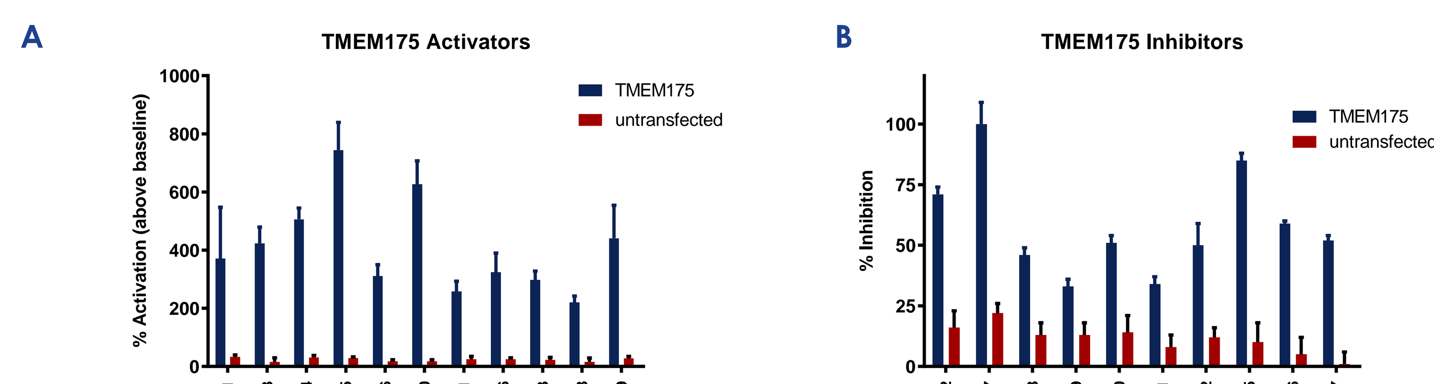
2 TMEM175 high-throughput automated patch clamp screening



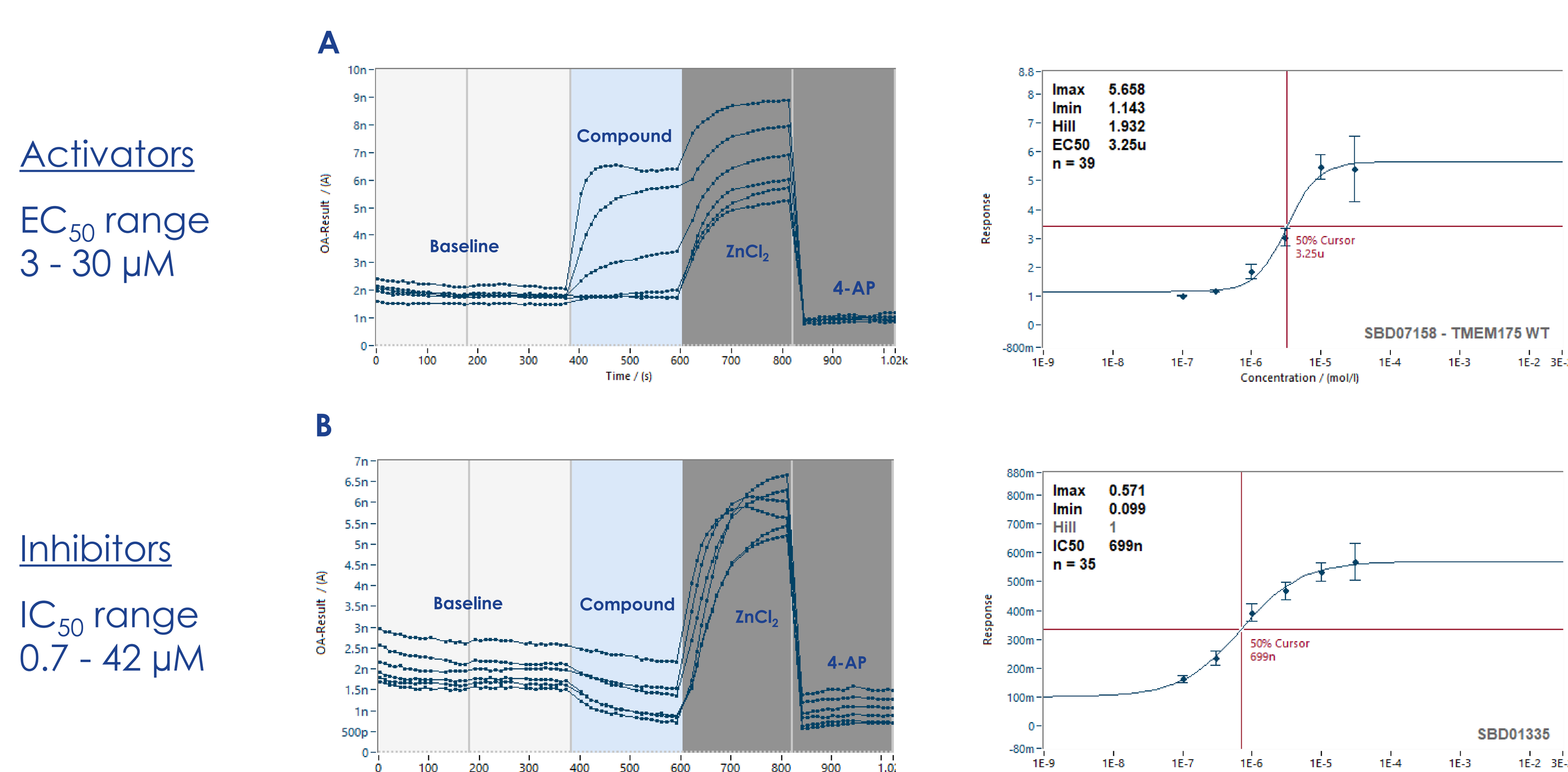
Application protocol. The application protocol consists of three additions of extracellular physiological solution for a minimum of 8 minutes, followed by an application of test compound for a minimum of three minutes. $ZnCl_2$ (500 µM) is then applied, before the channel is inhibited by a saturating concentration of 4-AP (1 mM).



A rapid and robust automated, high-throughput electrophysiology screening assay. SyncroPatch 384, high-throughput automated electrophysiology system was used to screen a library of 10,000 small molecules at a single concentration of 10 µM. Average success rate (A) and Z' factor (B) of the TMEM175 HTS assay. The screen yielded an average success rate of 82% (wells passing QC) and an average Z' factor of 0.77.

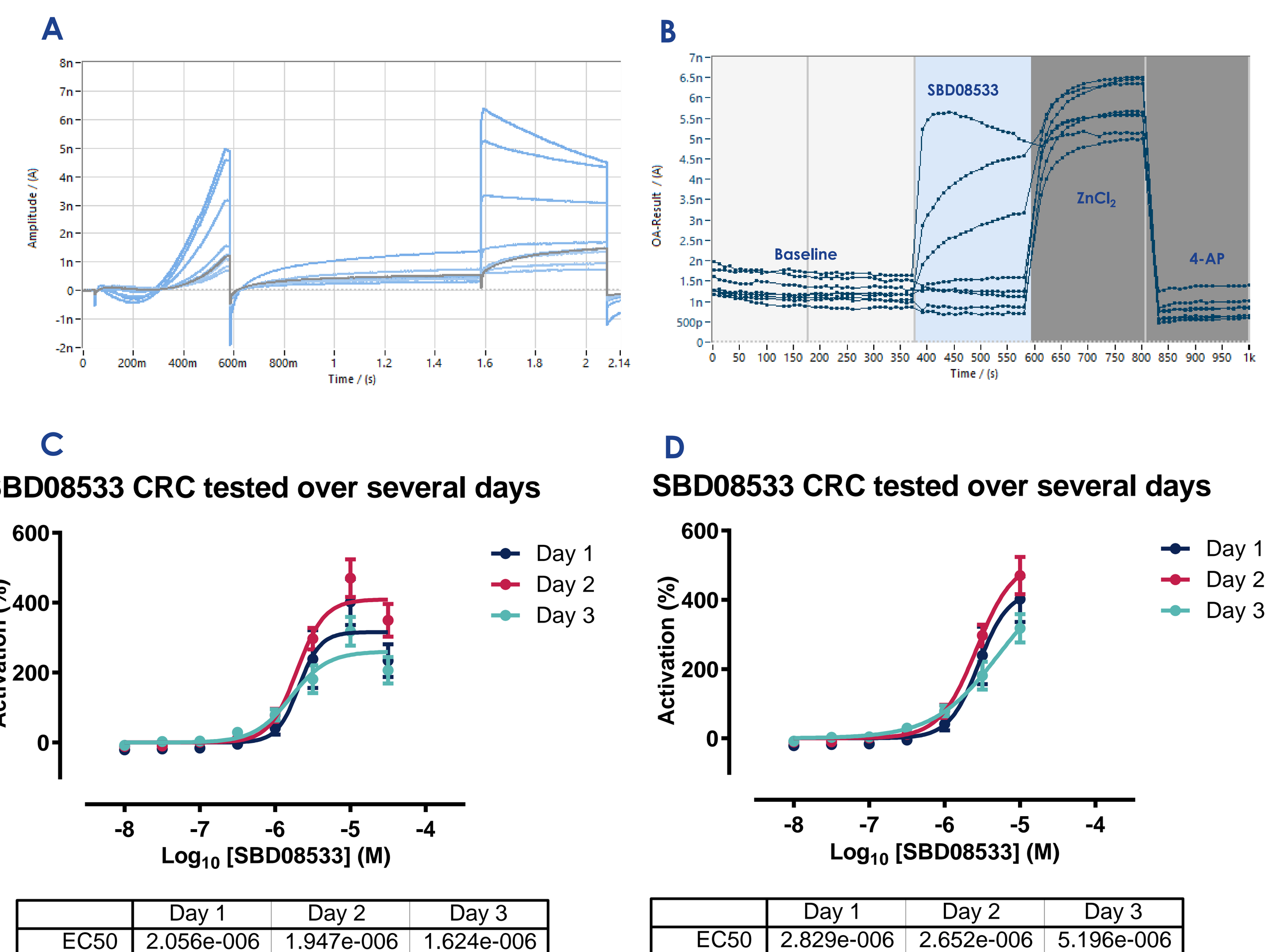


HTS Hit confirmation. % activation of the confirmed TMEM175 Activators (A) and inhibitors (B) against the TMEM175 (blue) and the untransfected HEK (red) cell lines.



TMEM175 Hit compound concentration response studies. Representative time courses and C-R curves of the confirmed TMEM175 Activators (A) and inhibitors (B) against the TMEM175 channel. The hit activators displayed EC_{50} values in the range of 3 - 30 µM while the hit inhibitors displayed IC_{50} values in the range of 0.7 – 42 µM.

3 TMEM175 activators and antagonists



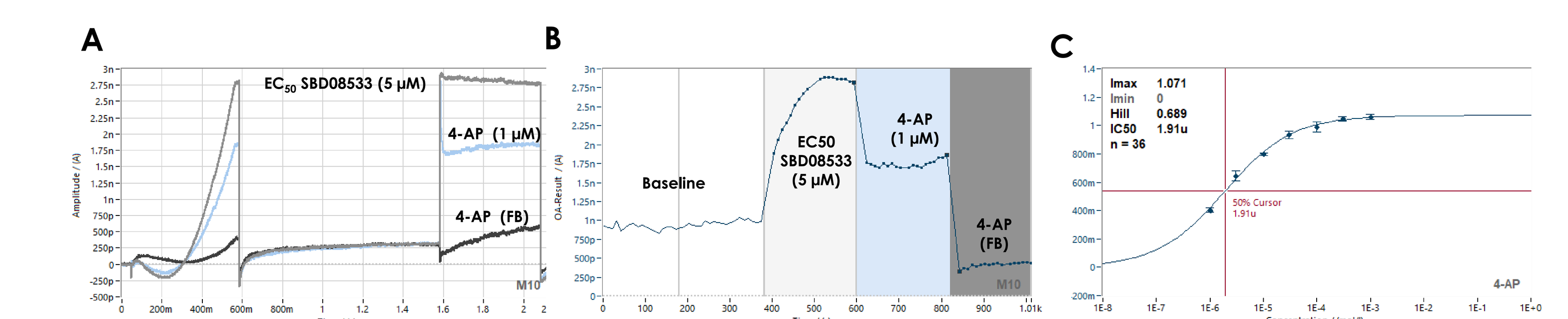
C:

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

D:

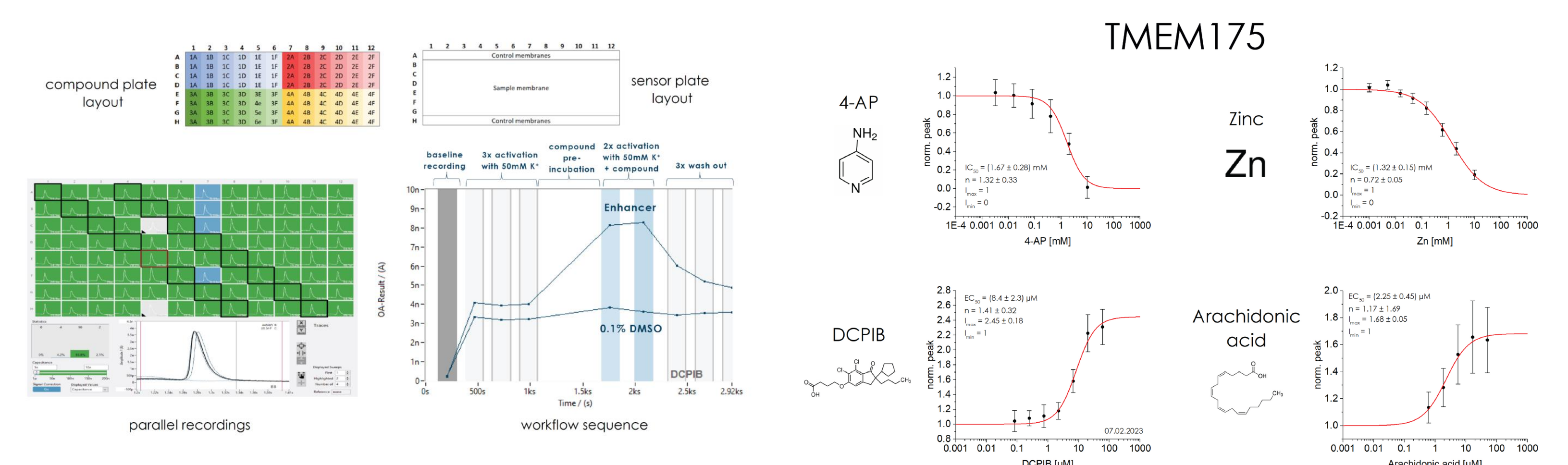
	Day 1	Day 2	Day 3
EC50	2.829e-006	2.652e-006	5.196e-006

SBD08533 – A reproducible TMEM175 Activator. Representative current traces (A) and time courses (B) of the hit activator SBD08533 against TMEM175. The application protocol consists of two additions of extracellular physiological solution for a minimum of 3 minutes each, followed by an application of SBDD08533 for a minimum of two minutes. $ZnCl_2$ (500 µM) is then applied before the channel is inhibited by a saturating concentration of 4-AP (1 mM). Normalised concentration response curves (C & D) showing the reproducible activation by SBD0833 tested over several days.



SBD08533 – Antagonist studies. Representative current traces (A), time course (B) and C-R curve (C) of the TMEM175 antagonist assay. Activation of TMEM175 by SBD08533 was inhibited in a concentration-dependent manner by reference inhibitor 4-AP.

4 Solid-supported membrane- (SSM-) based TMEM175 screening assay



New HTS technology for drug discovery on transporters and channels. The SURFE²R 96SE is a solid-supported membrane- (SSM-) based technology, which allows for measurements of slow and intracellular targets, not accessible in patch clamp in a 96-well format. Parallel target activation in presence of different compound concentrations reveals IC_{50} and EC_{50} values. Lysosomes were purified from HEK293 cells overexpressing TMEM175, kindly provided by SB Drug Discovery. Lysosomes were adsorbed to SURFE²R sensors according to Nanion´s standard protocol. 50 mM K^+ concentration gradients were used to trigger K^+ flux through TMEM175 at 0 mV and pH 7.6. H^+ conductivity assays are possible; native lysosomal pH gradients may be applied.

5 Conclusions

- TMEM175 stable cell line was characterized using the SyncroPatch 384 automated patch clamp system and used to develop and execute a high-throughput electrophysiology screening assay.
- This screening campaign successfully identified a number of active compounds with the ability to modulate TMEM175 in a concentration-dependent manner with EC_{50}/IC_{50} values in the low micromolar range.
- The successful development of a TMEM175 electrophysiology assay capable of identifying novel pharmacological tools will enable investigation of the role of this exciting target in normal physiology and in disease.
- An assay for TMEM175 on the SURFE²R 96SE was developed and dose-dependent signal enhancement and inhibition of TMEM175 currents from purified lysosomal membranes of the stable cell line overexpressing TMEM175 were successfully characterized. This approach allows for stable and robust recordings from proteins residing in organellar membranes with a throughput of up to 10'000 data points per day.

Recent TMEM175 Literature Review Update: Following generation of this data an article was published (Hu et al. Cell 2022) in which TMEM175 channel is suggested as a H^+ channel rather than a K^+ channel at low pH. We are now developing a H^+ assay to be used in conjunction with our K^+ assay. Visit our both for more information.