



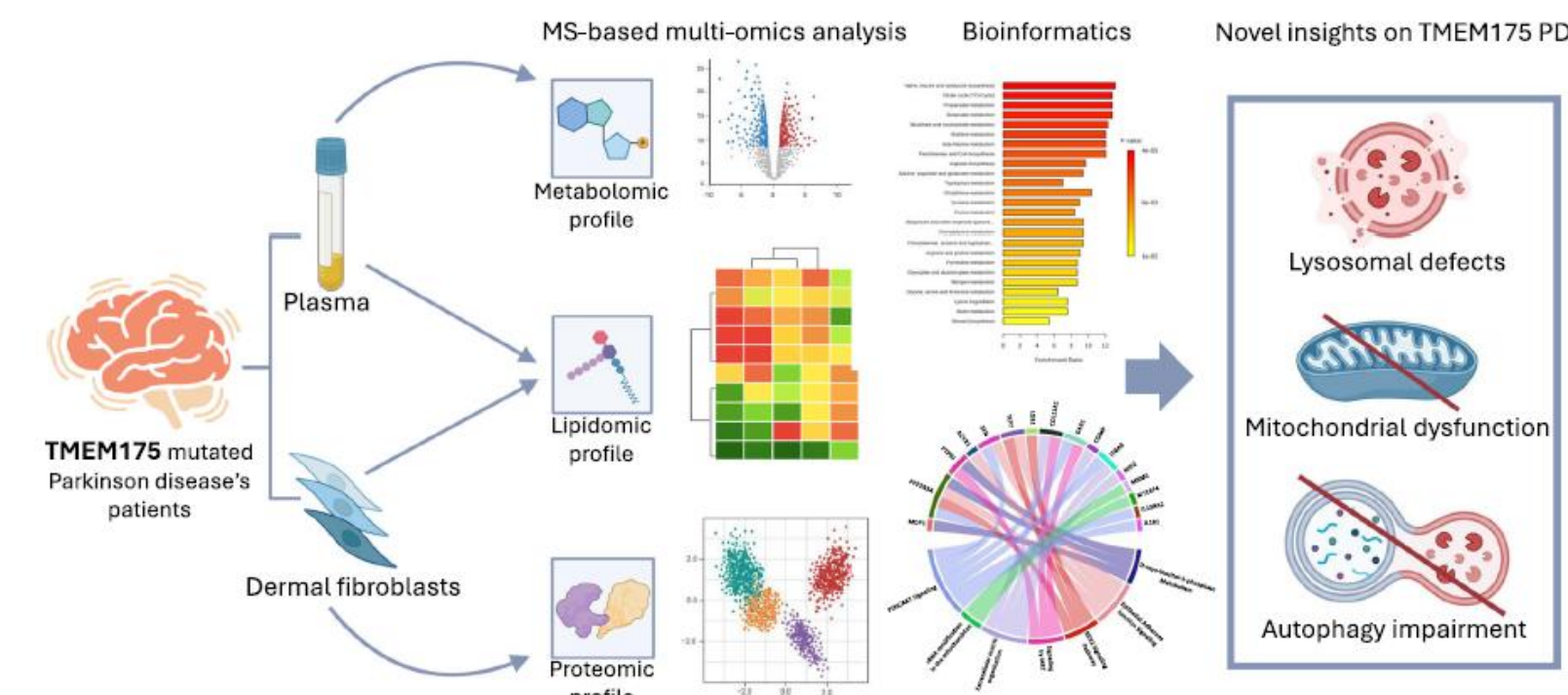
TMEM175 chronicles: a journey from High Throughput Screening to lysosome

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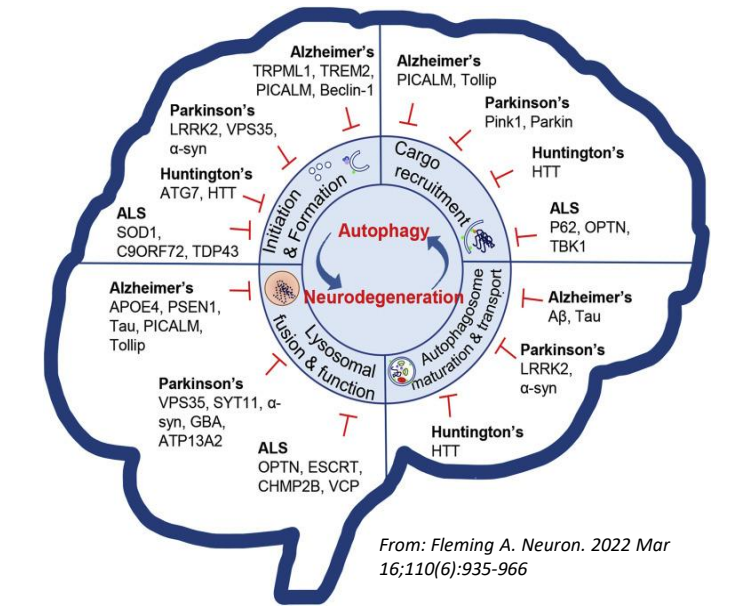
Introduction

Lysosomal dysfunction and impaired α -synuclein clearance are key drivers of Parkinson's disease, and genetic variants in TMEM175 increase disease risk by disrupting lysosomal pH, autophagy, and mitochondrial function. Partial TMEM175 dysfunction promotes protein accumulation, oxidative stress, and neurodegeneration, making TMEM175 activation a promising therapeutic strategy. To identify TMEM175 modulators, we developed an integrated platform combining high-throughput screening with mechanistic assays. This included a scalable automated lysosomal patch-clamp system and, importantly, single-channel electrophysiology on lysosomes isolated from iPSC-derived dopaminergic neurons carrying wild-type or disease-associated TMEM175 variants. This platform enables precise pharmacological characterization of TMEM175, bridging high-throughput drug discovery with detailed functional analysis and supporting the development of novel therapies for Parkinson's disease.



Summary

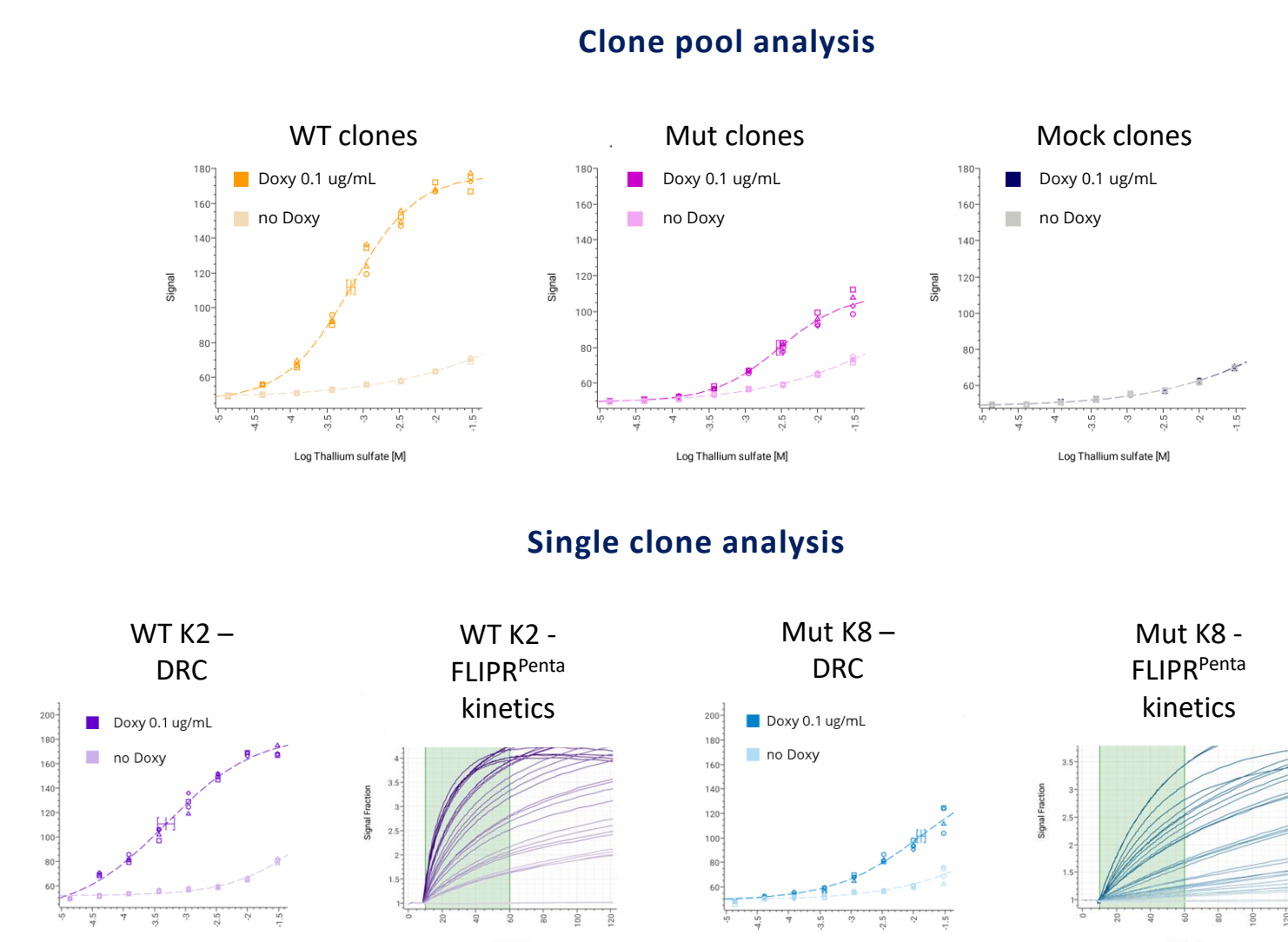
- Intracellular ion channels are known to play an essential role in various signaling pathways for health and disease
- Targeting lysosomal dysfunction has emerged as a potential therapeutic approach for neurodegenerative disorders such as Parkinson's Disease (PD) and Amyotrophic Lateral Sclerosis (ALS) with a particular interest in the study of function and pharmacological modulation of lysosomal ion channels
- Large interest in exploring intracellular ion channels as therapeutic targets has grown tremendously indicating a need for high-throughput electrophysiology assays for drug discovery, however so far lacking the possibility to collect data from native Lysosomes
- The present study shows data registered from isolated lysosomes on an HTS automated patch-clamp device combined with an innovative lysosomal patch-clamp on iPS derived neurons



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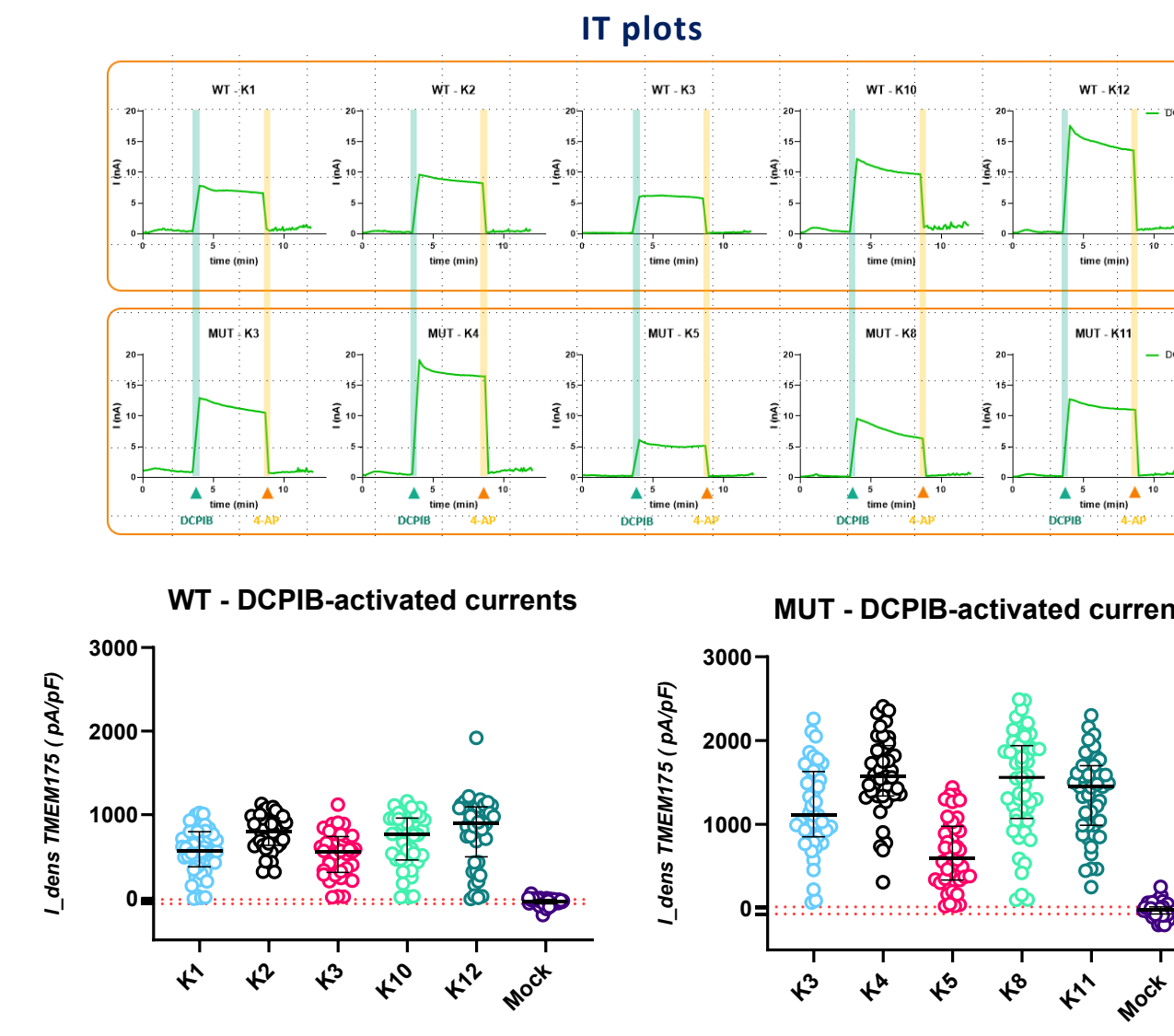
TMEM175 overexpressing Cell lines generation and clone validation

Cell line generation and clone selection using thallium response



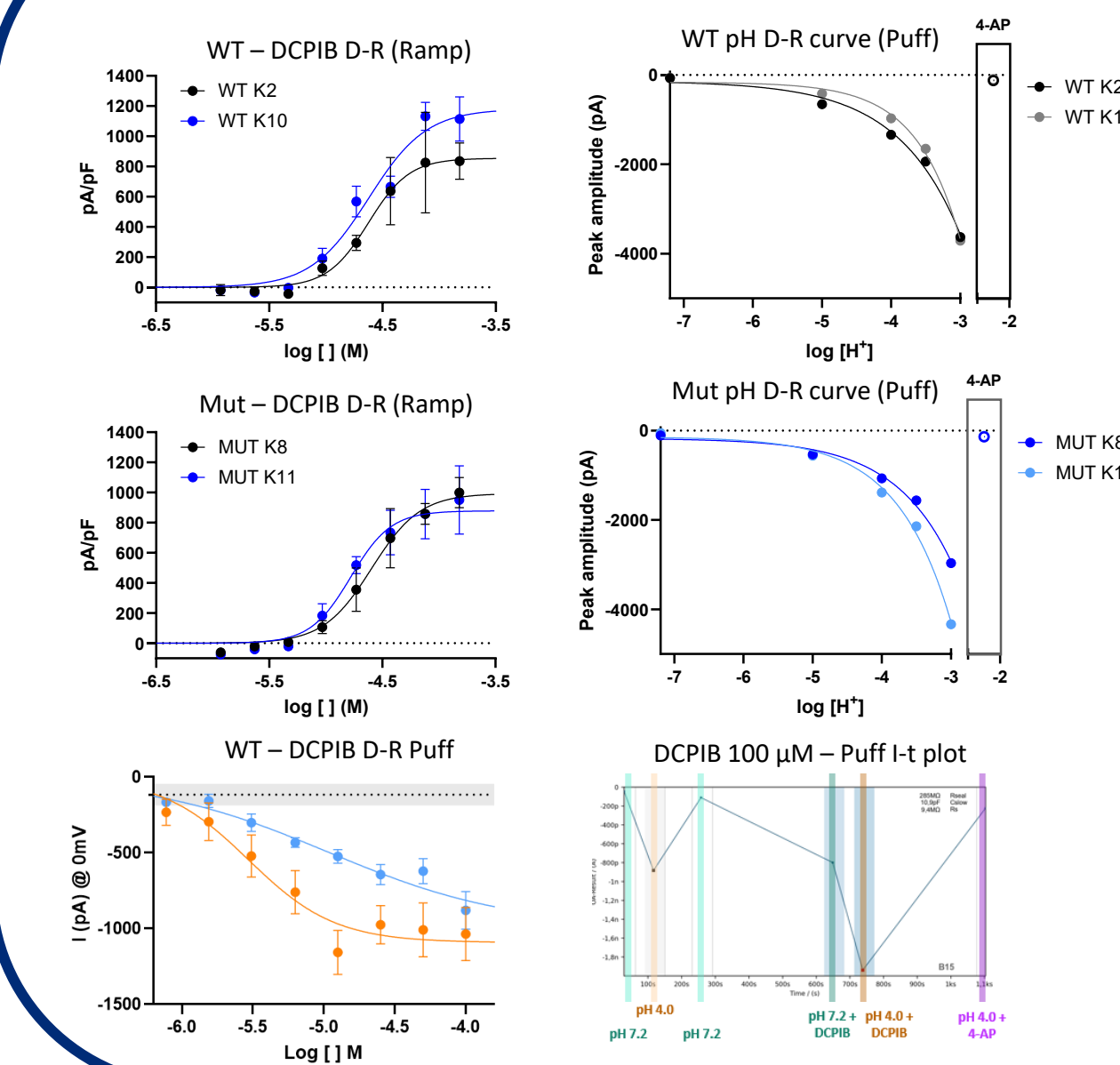
- Both pools displayed a good activation in response to thallium sulphate.
- Slight activation was also detected in the mock and not induced cells, highlighting that the signal is specific, as it differs significantly from that of the induced clones
- 12 well performing clones were further characterized and the best 6 responder clones of each cell line were selected and tested at SP384.

Clone selection at SP384



- A voltage ramp was used, and the current amplitude was measured at +100mV.
- All tested clones responded to 100 μ M DCPIB and were blocked by 2mM 4-AP with a good overall success rate (catch, seal, expression).
- All the clones showed an outward current compatible with TMEM175 and statistically different from mock cells.
- WT K2 and K10, Mut K8 and K11 were chosen for further characterization.

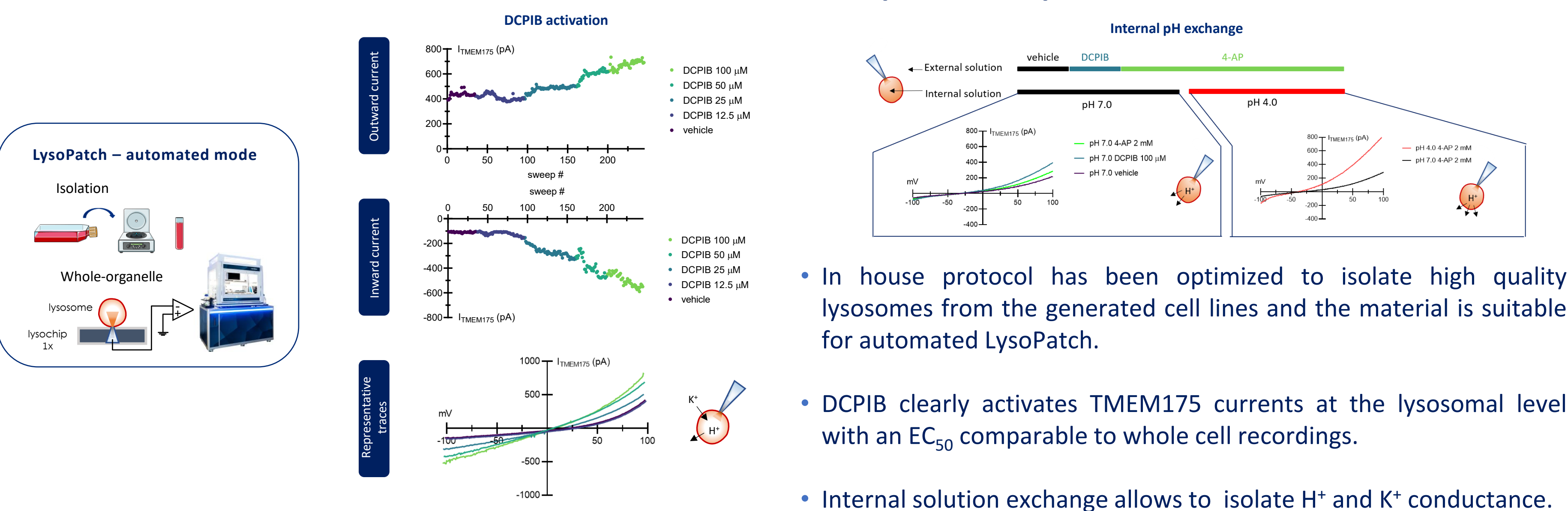
Assay optimization at SP384



- DRC for DCPIB are reliable for all the investigate clone and the EC_{50} values are comparable with what has been observed previously.
- Taking advantage of the puff method (Nanion) the response to pH was recorded for the different clones.
- A significative increase in of peak current (proton flux) was recorded, as expected, upon acidification.
- The DRC for DCPIB was also evaluated with the puff method at pH 7.2 and 4.0. Then net proton current increases upon increasing DCPIB concentration.

TMEM175 at the lysosomal level

Automated patch clamp

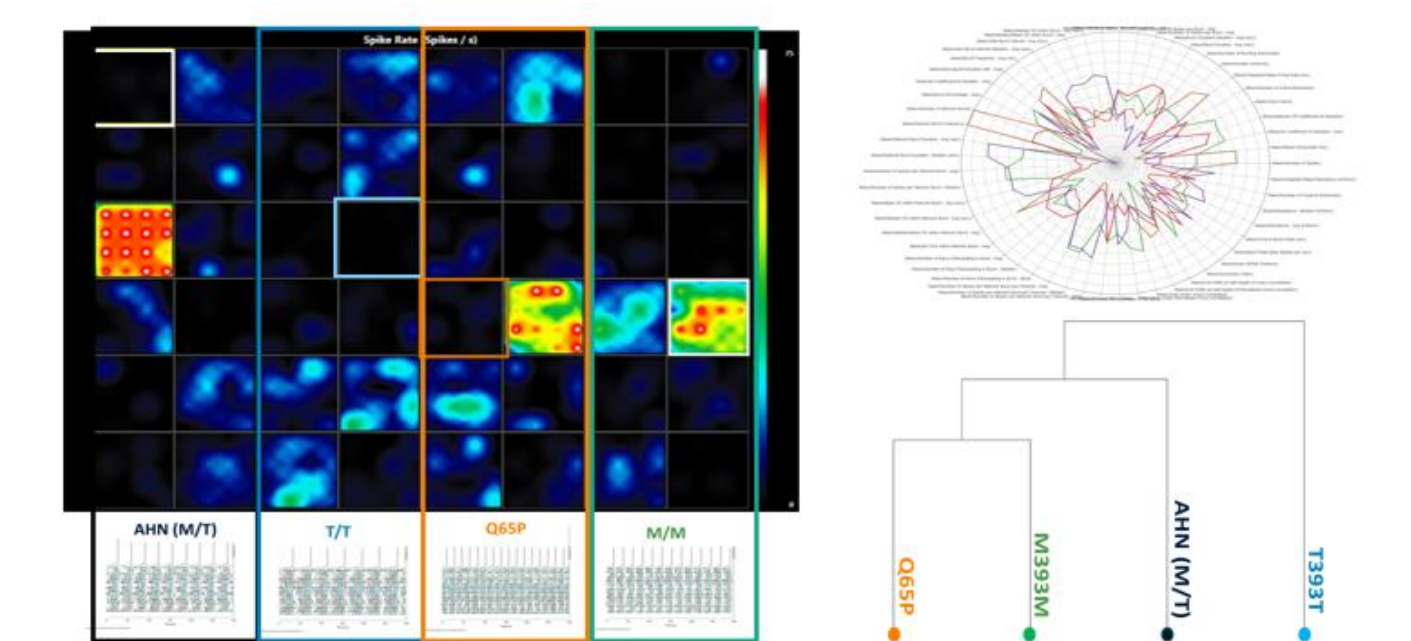


- In house protocol has been optimized to isolate high quality lysosomes from the generated cell lines and the material is suitable for automated LysoPatch.
- DCPIB clearly activates TMEM175 currents at the lysosomal level with an EC_{50} comparable to whole cell recordings.
- Internal solution exchange allows to isolate H^+ and K^+ conductance.

iCell® DopaNeurons (FCDI)

Lysosome Patch Clamp

- DopaNeurons with different TMEM175 variants were cultured for 14 days and analyzed by lysosomal patch-clamp.
- Whole-lyso and lyso-attached recordings showed consistent alterations among genotypes.
- Single channel conductance (pS) calculated from the slope of the IV relationship between 60 mV and 100 mV did not show significant difference among the 4 genotypes
- The Q65P variant showed more channel events at similar amplitude, suggesting higher TMEM175 expression on lysosomal membranes, consistent with whole-lyso data.



Multielectrode Array (MEA)

The multi-electrode array (MEA) platform was used to study iCell® DopaNeurons network activity: dendrogram clustering showed the longest distance between Q65P and T393T mutants, while AHN and M393M clustered closely, supporting single-channel findings.

Conclusions

- Stable cell lines expressing WT and mutated TMEM175 were first selected using a thallium fluorescence assay and further characterized electrophysiologically. All tested clones responded to the TMEM175 activator DCPIB, acidic pH and were blocked by 4-AP with a good overall success rate (catch, seal, expression).
- The initial studies at the plasma membrane level were extended to the lysosomal membrane using both manual (data not shown) and automated patch-clamp techniques, enabling precise measurement of TMEM175-mediated ionic currents within isolated lysosomes.
- In addition, lysosomal function was examined in iPS-derived dopaminergic neurons (iCell® Dopaneurons, FCDI), offering a physiologically relevant model to study TMEM175 in the context of neurodegenerative conditions. This approach provides a unique opportunity to integrate insights into TMEM175 function with disease-relevant cellular models, further advancing our understanding of its role in lysosomal health and its potential as a therapeutic target.

We kindly acknowledge FUJIFILM Cellular Dynamics for their support and for the kind provision of the iPS-derived cell line used in this work

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