



# TMEM175 CHRONICLES: A JOURNEY FROM HIGH THROUGHPUT SCREENING TO LYOSOSOME

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## ABSTRACT

Lysosomal abnormalities are a hallmark of neurodegenerative diseases, with common features including altered lysosomal pH, impaired enzyme activity, and defects in lysosomal trafficking. TMEM175, a member of the transmembrane protein family, has garnered considerable attention for its critical role in lysosomal function.

This study investigates TMEM175 using a diverse set of methodologies, from high-throughput screening to detailed single-channel recordings on lysosomal membranes.

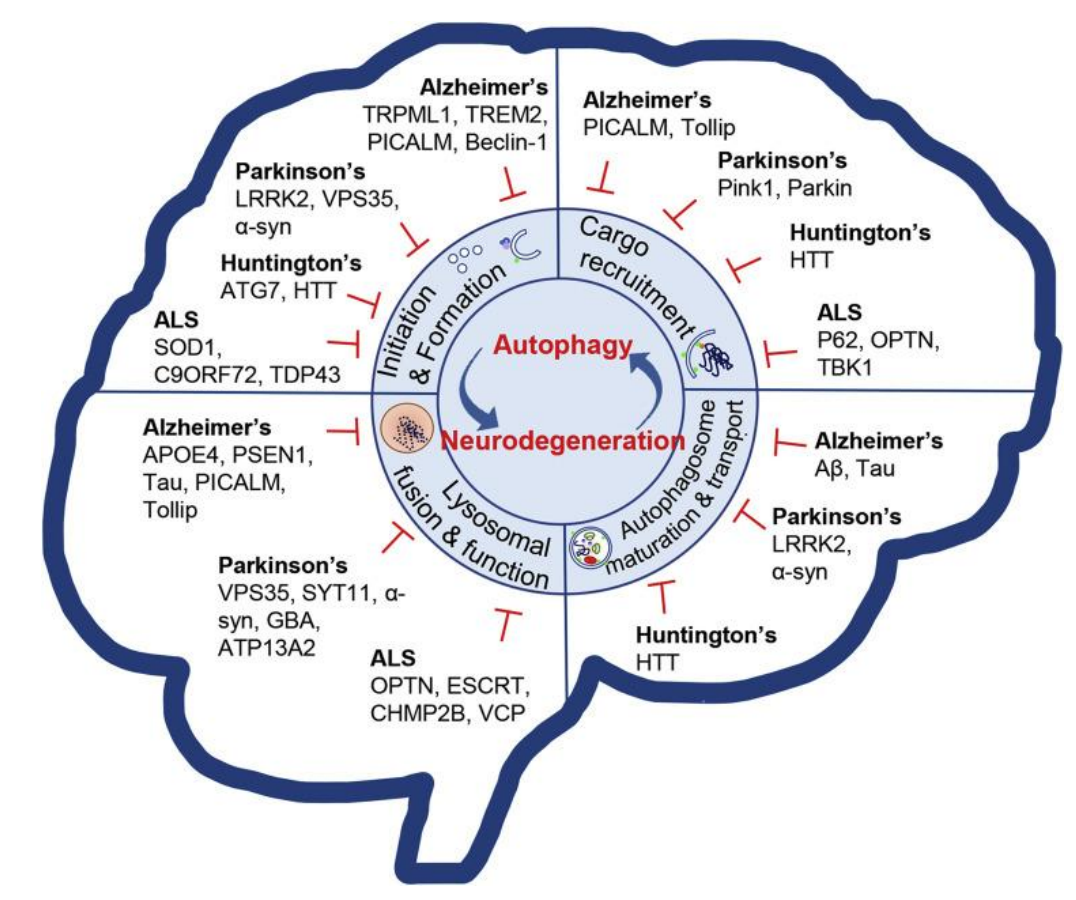
We first generated stable cell lines expressing TMEM175 and identified potential modulators via a thallium fluorescence assay. Electrophysiological characterization followed, initially focusing on the plasma membrane and later extending to the lysosomal membrane. Both manual and automated patch-clamp techniques were employed to precisely measure TMEM175-mediated ionic currents within isolated lysosomes. While manual patch-clamping provides valuable insights into organellar function, its labor-intensive nature and limited scalability for large-scale drug screening presented significant challenges. To address these, we developed a robust lysosomal high-throughput screening (HTS) platform using the SyncroPatch system (Nanion), which dramatically enhances throughput by enabling compound testing on a 384-well array.

Additionally, lysosomal function was evaluated in iPSC-derived dopaminergic neurons, providing a physiologically relevant model to study TMEM175 in the context of neurodegenerative diseases. This approach bridges mechanistic insights into TMEM175 function with disease-relevant cellular models, advancing our understanding of its role in lysosomal health and its potential as a therapeutic target. In summary, this comprehensive study underscores the significance of TMEM175 across multiple experimental paradigms, from high-throughput platforms to intricate lysosomal analyses, thereby advancing translational research and enhancing our understanding of neurodegenerative disease mechanisms.

## SUMMARY

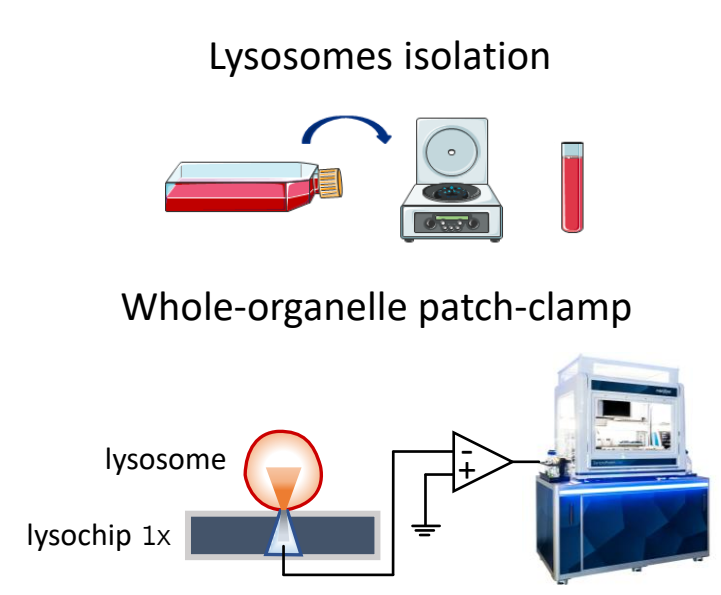
- Intracellular ion channels are crucial for various signaling pathways that govern both health and disease.
- Targeting lysosomal dysfunction has become a promising therapeutic approach for neurodegenerative disorders such as Parkinson's Disease (PD) and Amyotrophic Lateral Sclerosis (ALS), with a particular focus on understanding and modulating the function of lysosomal ion channels.
- The growing interest in intracellular ion channels as therapeutic targets underscores the need for high-throughput electrophysiology assays in drug discovery. However, the ability to gather data from native lysosomes has remained a significant challenge.
- This study addresses this gap by presenting data obtained from isolated lysosomes using an HTS automated patch-clamp device. Additionally, an innovative lysosomal patch-clamp technique applied to iPSC-derived neurons is employed, providing new insights into lysosomal ion channel function and opening avenues for more efficient drug discovery.

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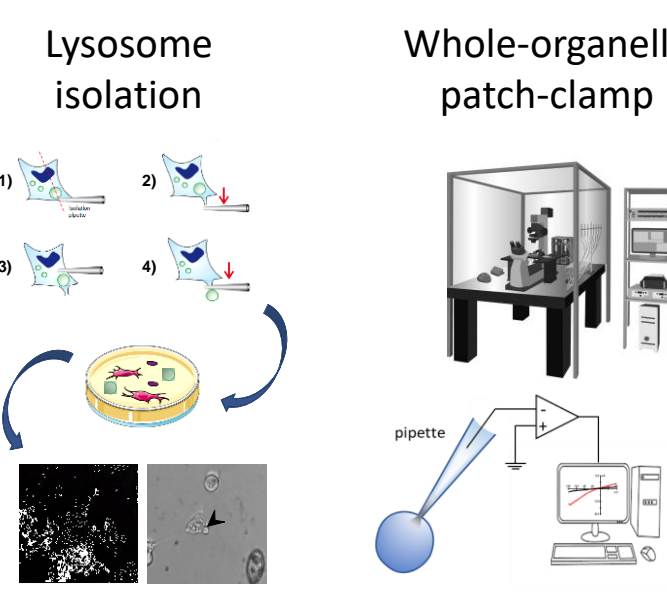


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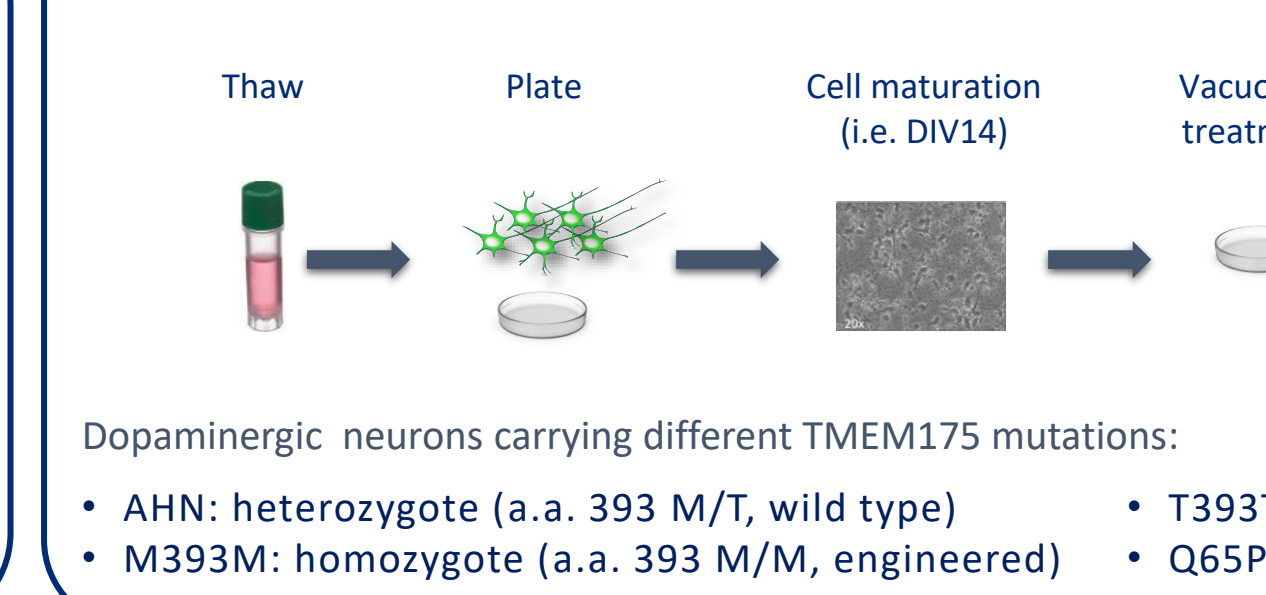
### LysoPatch – automated mode



### LysoPatch – manual mode



### LysoPatch on iCell® DopaNeurons



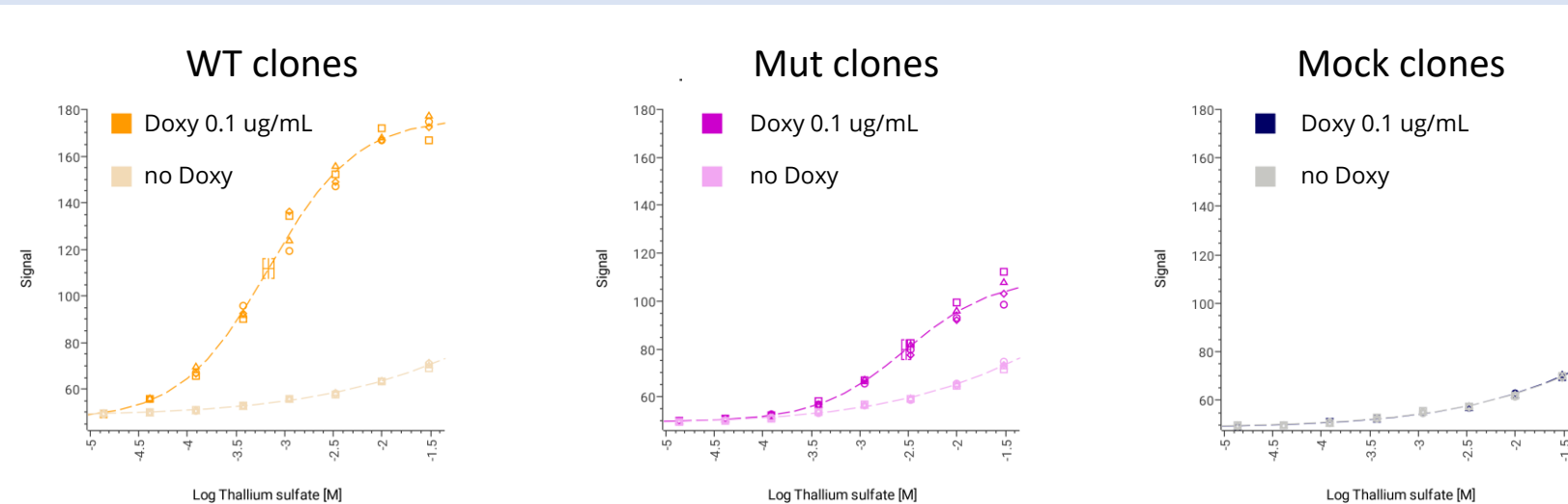
Dopaminergic neurons carrying different TMEM175 mutations:

- AHN: heterozygote (a.a. 393 M/T, wild type)
- M393M: homozygote (a.a. 393 M/M, engineered)
- T393T: homozygote LOF (a.a. 393 T/T, engineered)
- Q65P: heterozygote GOF (a.a. 393 M/M, a.a. 65 Q/P, engineered)

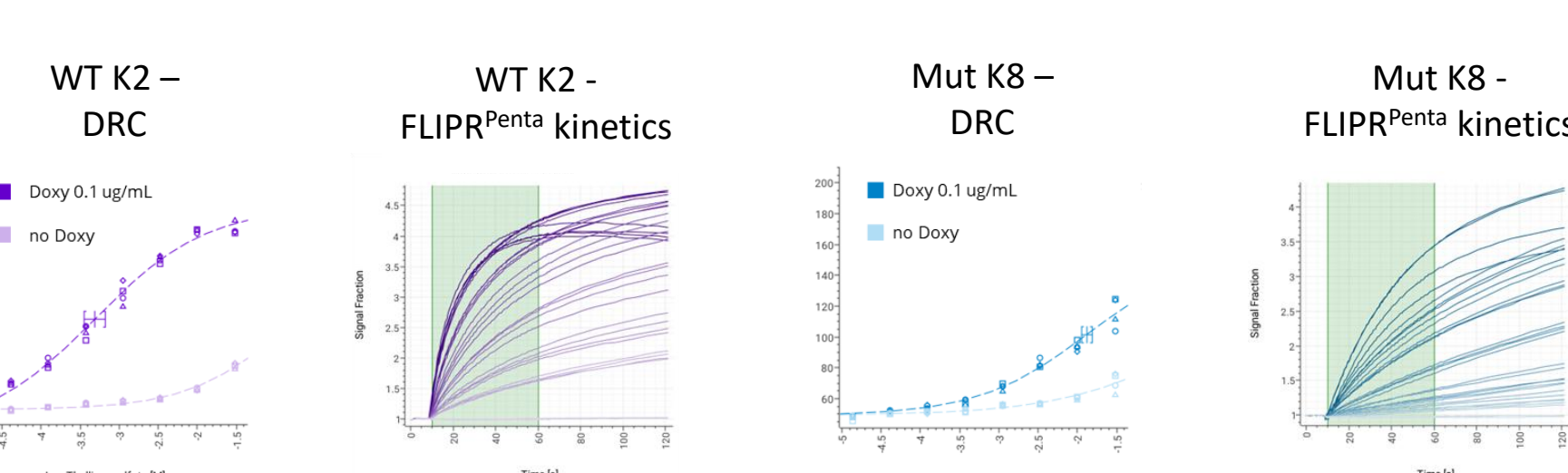
## TMEM175 overexpressing Cell lines generation and clone validation

### Cell line generation and clone selection using thallium response

#### Clone pool analysis

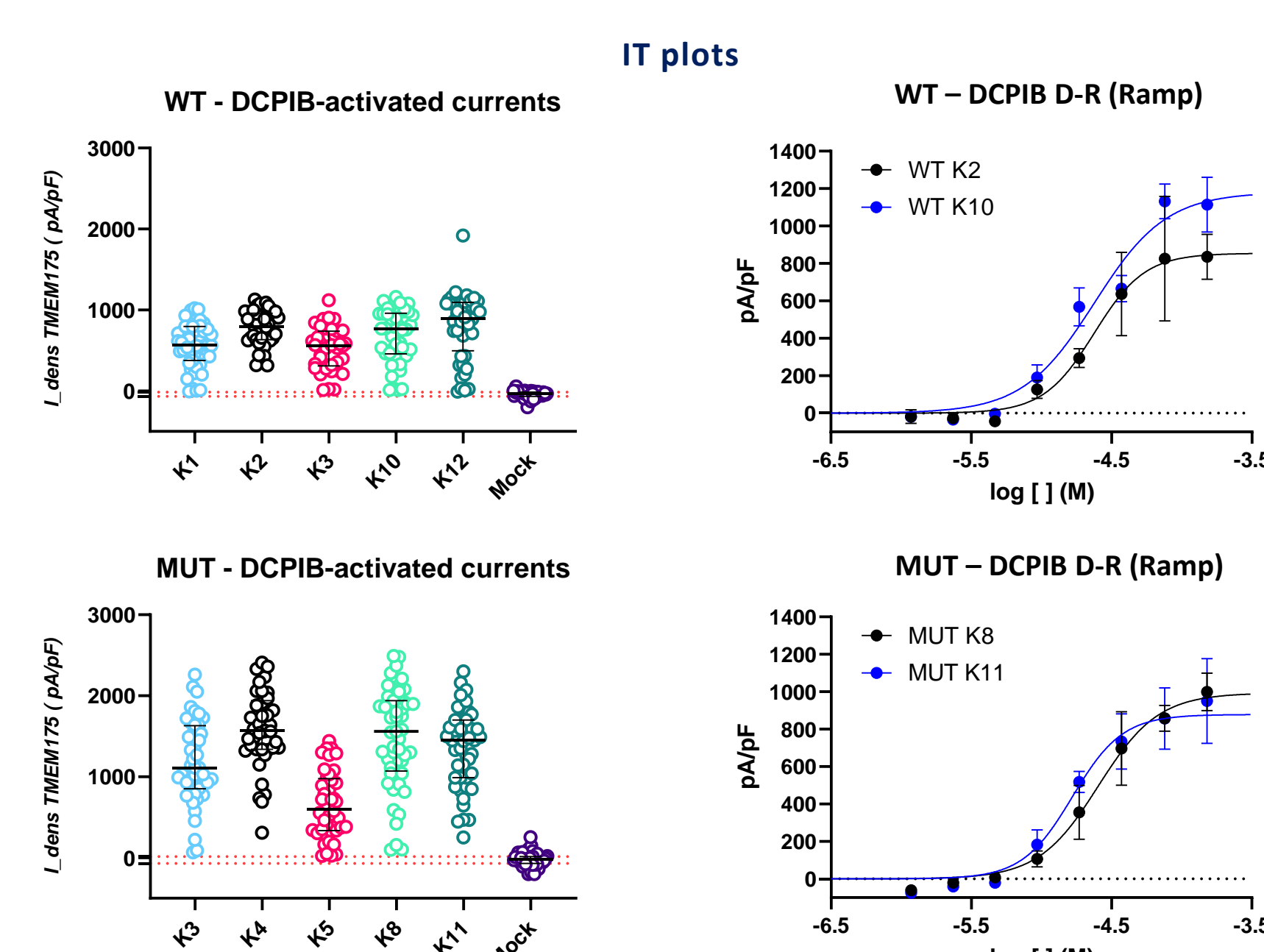


#### Single clone analysis



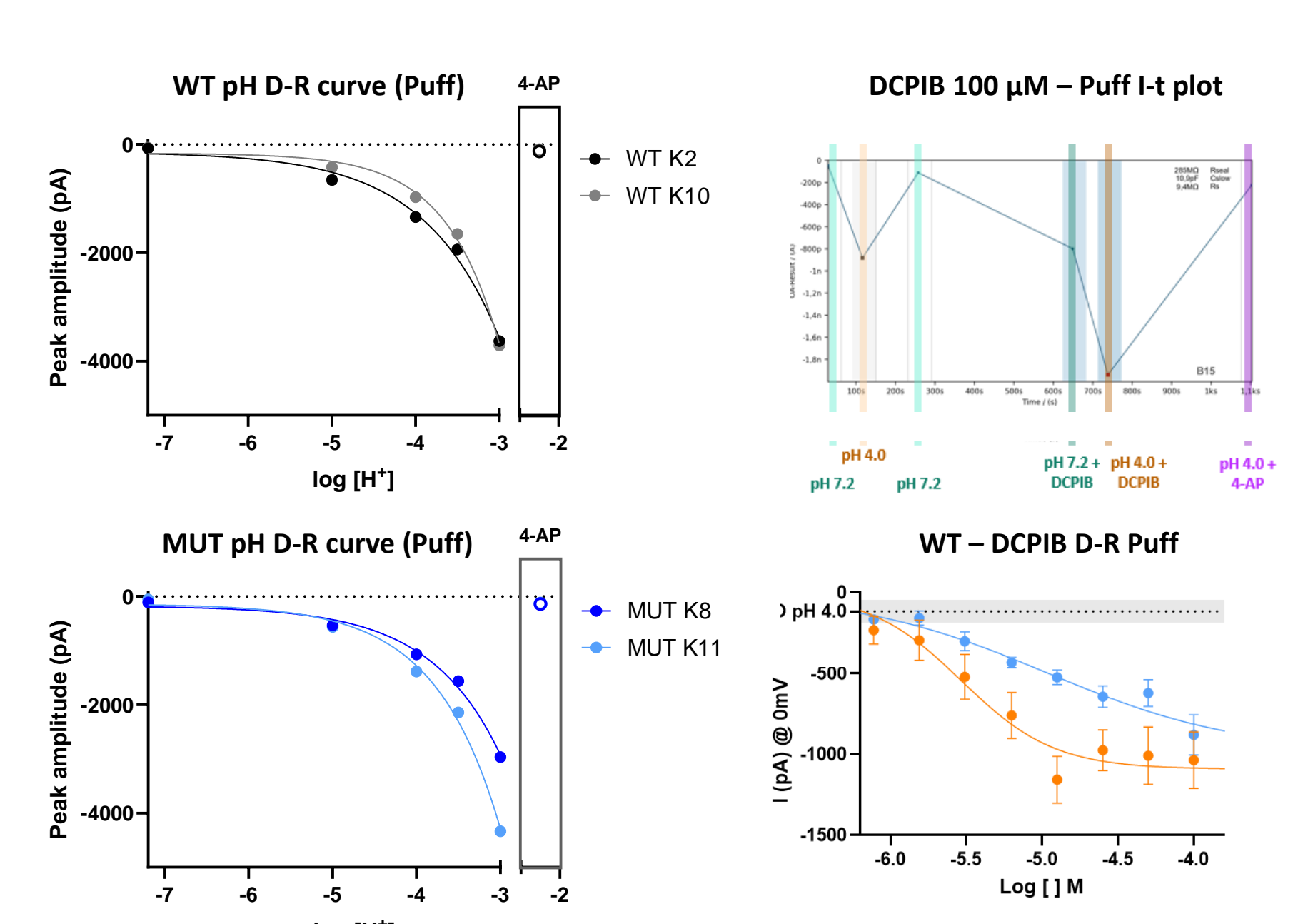
- Both pools displayed a good activation in response to thallium sulphate.
- Only a slight activation was detected in the mock and not induced cells; the signal significantly different from that of the induced clones, highlighting its specificity.
- 12 well performing clones were further characterized and the best 6 responder clones of each cell line were selected and tested at SP384.

### Clone selection at SP384



- A voltage ramp protocol was used to evaluate the current amplitude at +100mV.
- All tested clones responded to 100 μM 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

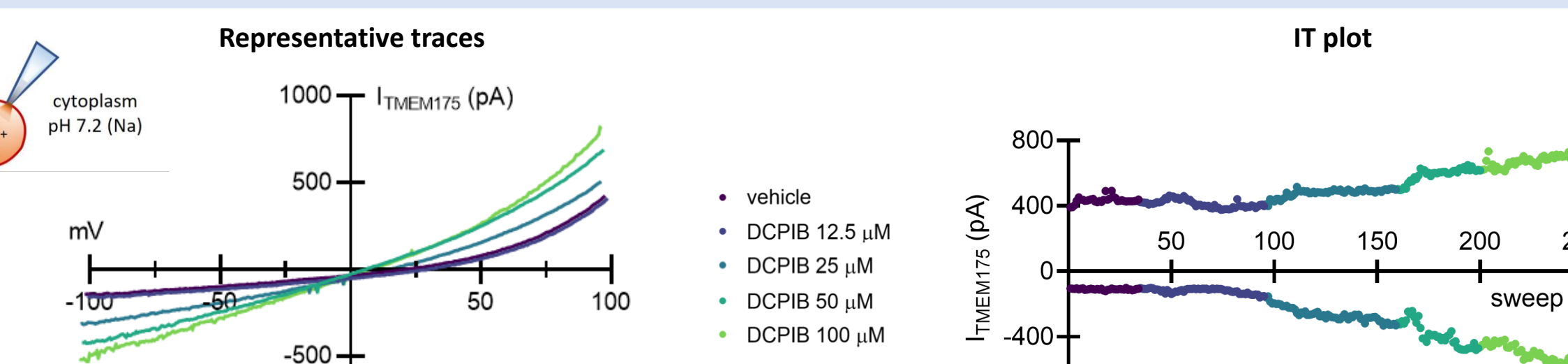


- DRCs for DCPIB are reliable in all the investigated clones and the EC<sub>50</sub> values are comparable with what observed previously.
- A significant peak current increase (proton flux) was recorded upon acidification (puff method - Nanion).
- The DRC for DCPIB was also evaluated using the puff method at pH 7.2 and 4.0. The net proton current responds to increasing DCPIB concentrations.

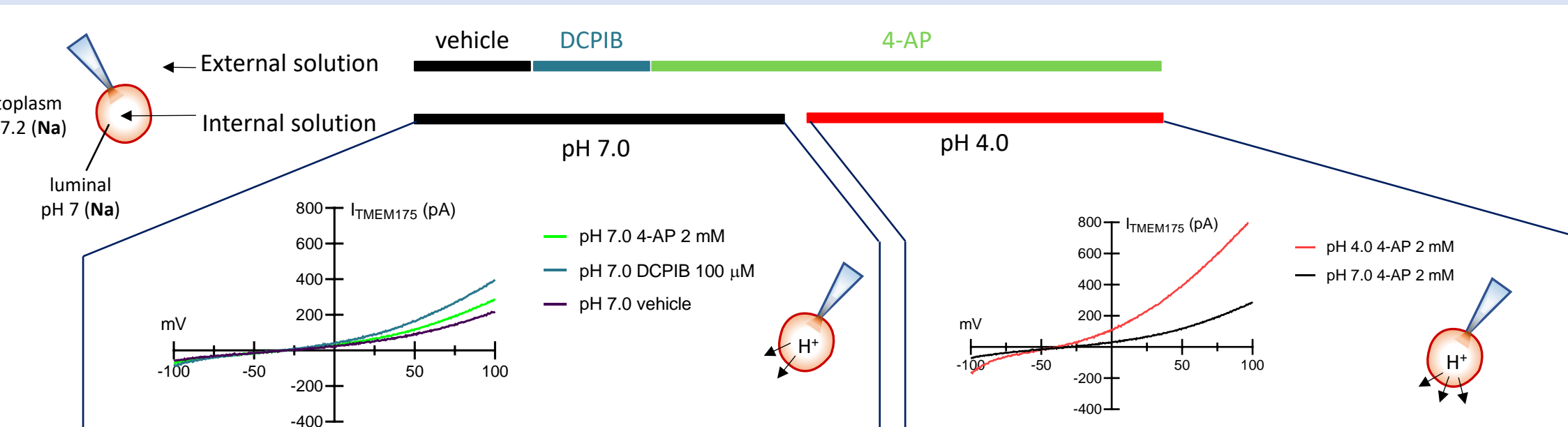
## TMEM175 at the lysosomal level

### Automated patch clamp

#### DCPIB activation



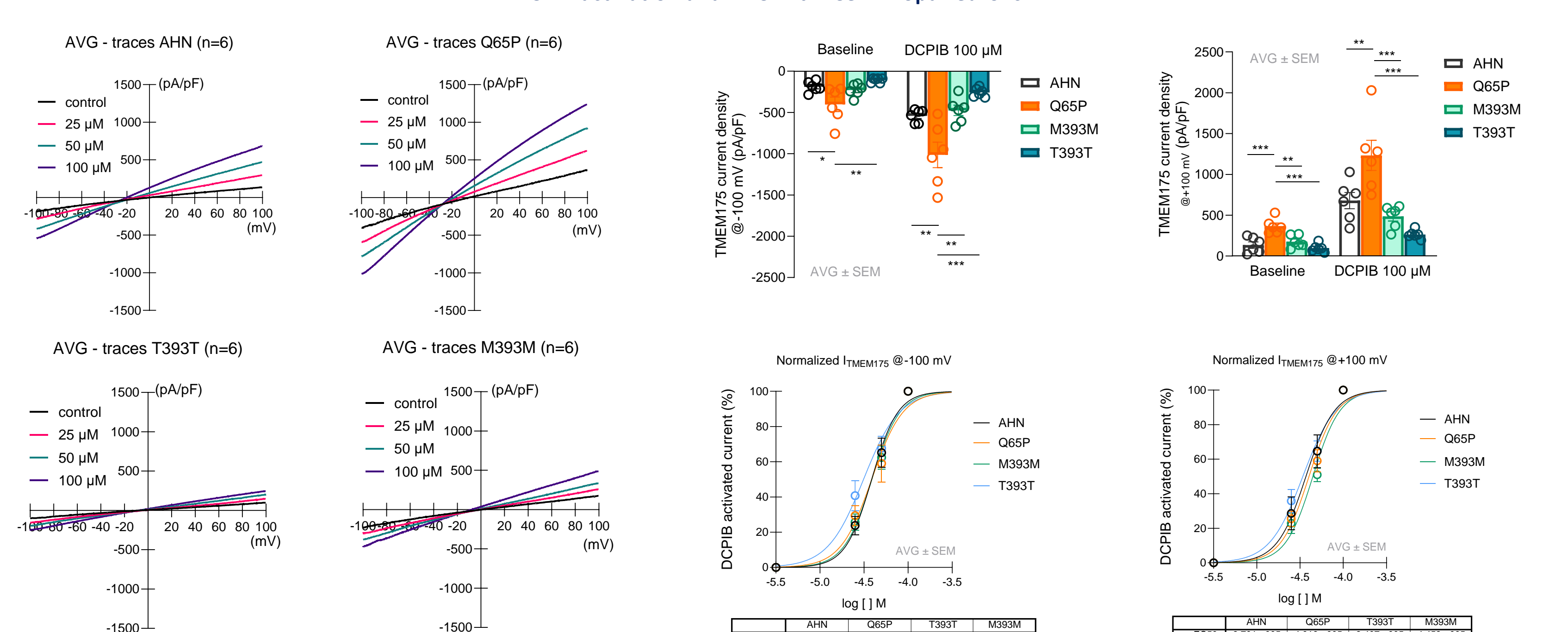
#### Internal pH exchange



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

### iPSC-derived dopaminergic neurons

#### DCPIB activation and DRC with iCell® DopaNeurons



- iCell® DopaNeurons with different TMEM175 mutations associated with Parkinson's Diseases and other α-synucleinopathies were cultured up to 14 days.
- The lysosomes of the four cell lines were patched manually and the response to different DCPIB concentrations were recorded.
- The DRCs to DCPIB are comparable in all conditions (cell line, current read-out) and similar to what already observed in HEK-cells.
- TMEM175 Q65P mutant line showed the highest activation, while the homozygous M393M Mutant (M393M) showed as expected, the lowest activation.

## Conclusions

- Stable cell lines expressing wild-type (WT) and mutated TMEM175 were successfully selected using a thallium fluorescence assay and further characterized electrophysiologically. All tested clones exhibited consistent responses to the TMEM175 activator DCPIB, acidic pH, and were effectively blocked by 4-AP, with a high success rate for catch, seal, and expression.
- Building on initial studies at the plasma membrane level, we extended our investigations to the lysosomal membrane using both manual and automated patch-clamp techniques. These methodologies enabled precise measurement of TMEM175-mediated ionic currents within isolated lysosomes.
- Furthermore, lysosomal function was evaluated in iPSC-derived dopaminergic neurons (iCell® DopaNeurons, FCDI), providing a physiologically relevant model to explore TMEM175 in the context of neurodegenerative diseases. This approach offers a unique opportunity to integrate mechanistic 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 for neurodegenerative conditions.