Supplementary Information

Electrophysiology assay for hTRPML3 we generated four hTRPML3 mutation H283A and whole-cell current recording result (Fig. 1) Previous studies show that TRPML3 channels are inhibited by low luminal pH and that a histidine (H283) in the PMD is critical for this inhibition. We reproduced these results, but by using a solution-exchange protocol that more closely mimics the ionic conditions of normal and neutralized/damaged lysosomes, we found that low luminal pH actually produced two different modes of inhibition. In whole-cell recordings of WT TRPML3 channels, changing the bath solution from a 160 mM Na+ pH 7.4 solution (similar to extracellular solution) to a 160 mM Na+ pH 4.6 solution (close to normal lysosome condition) fully inhibited the currents (Fig. 1). This inhibition was largely irreversible as long as the channels were continuously bathed in the high Na+, neutral pH solution; thus, there is a long-lasting Inhibition Memory.

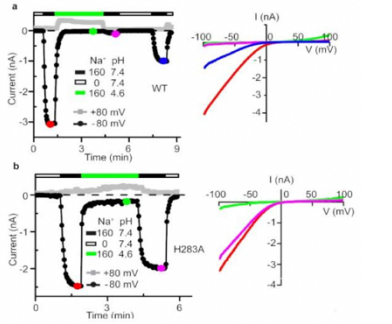

Fig.1 Two modes of low pH inhibition and the "Inhibition Memory".(a) Time course (left) and I-V relationships (right) of WT (a) and H283A (b) channel currents in response to low pH.

Protein purification and amphipol exchange for hTRPML3

hTRPML3 proteins were expressed in SF9 insect cells. Protein was purified with detergent and finally exchanged in amphipol. Gel filtration and SDS-PAGE results indicate that hTRPML3 samples are homogenous and good enough for cryo-EM study (Fig. 2).

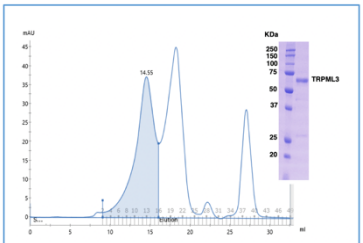

Fig.2 Gel filtration and SDS-PAGE analysis of TRPML3 samples.

Image acquisition on F20 and processing for hTRPML3

We prepared cryogenic grids using vitrobot machine (FEI) for hTRPML3 proteins, and collected a 448-micrograph dataset on F20 microscope. After image processing using Relion and cryoSPARC, we got very promising 2D classification result for both samples. Representative views from 2D classification showed clear 4-fold symmetry feature for hTRPML3. Through 3D refinement and post-processing, we were able to refine hTRPML3 map to 3.82 Å resolution (Fig. 3) .

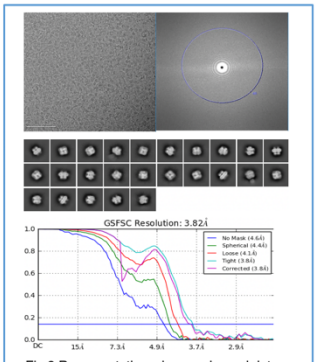

Fig.3 Representative micrographs and data processing for hTRPML3

Grid availability and session request

We already have back-up grids for hTRPML3 with PIP2 in different pH. They are available for data collection on Titan Krios. For session request, we would like to apply for 4 sessions with 24 hours for each session.