Supplementary Information

Electrophysiology assay for Truncated TRPML2

We did patch-clamp to record the activity of TRPML2. Whole-cell current recording result (Fig. 1) showed that the addition of agonist ML-SA1 and ML2-SA1 can activate the TRPML2 channel at 10 μM concentration.

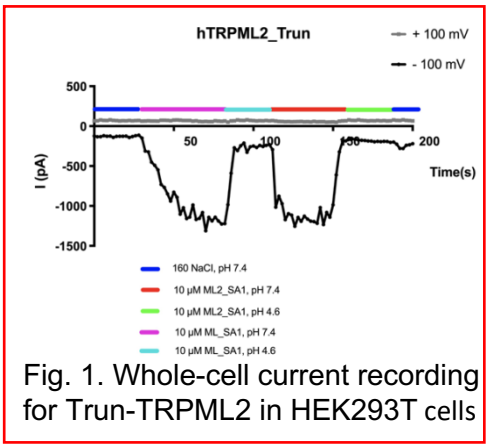

Fig. 1. Whole-cell current recording for Trun-TRPML2 in HEK293T cells

Protein purification and nanodisc reconstitution for Trun-TRPML2 apo and agonist-bound states

Truncated hTRPML2 protein was expressed in SF9 insect cells. Protein was purified with detergent and reconstituted into nanodisc with MSP1E3 protein. Agonist-bound sample was generated by incubating the protein and agonist together overnight for nanodisc reconstitution. Gel filtration and SDS-PAGE results indicate that both apo and agonist-bound TRPML2 samples are homogenous and good enough for cryo-EM study (Fig. 2).

Image acquisition and processing for Trun-TRPML2 apo and agonist-bound states

We prepared cryogenic grids using vitrobot machine (FEI) for Trun-TRPML2 apo and agonist-bound samples. With apo TRPML2, we were able to get a 3.37 Å resolution structure on Krios microscope (Fig. 3). With ML2-SA1 bound TRPML2, we collected a 2402-micrograph dataset on Glacios microscope. After image processing using Relion and cryoSPARC, we got very promising 2D classification result for ML2-SA1 bound sample. Representative views from 2D classification showed clear 4-fold symmetry feature for ML2-SA1 bound TRPML2. (Fig. 4b). Through 3D refinement and post-processing, we were able to refine ML2-SA1 bound TRPML2 map to 6.64 Å resolution (Fig. 4c)

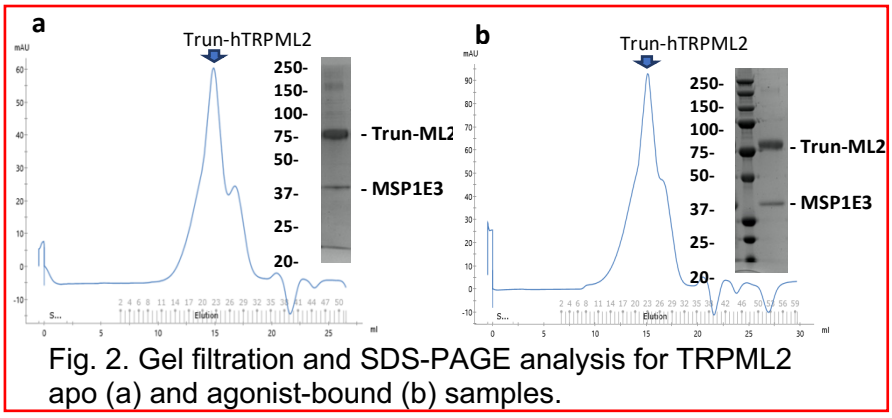

Fig. 2. Gel filtration and SDS-PAGE analysis for TRPML2 apo (a) and agonist-bound (b) samples.

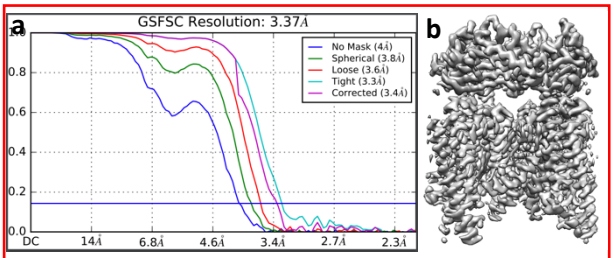

Fig. 3. Cryo-EM map of apo Trun-TRPML2

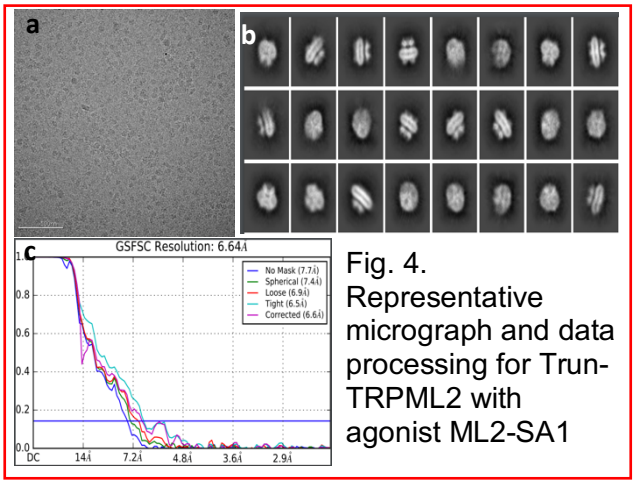

Fig. 4. Representative micrograph and data processing for Trun-TRPML2 with agonist ML2-SA1

Grid availability and session request

We have back-up grids for both ML-SA1 and ML-SA1 bound TRPML2. They are available for data collection on Titan Krios. For session request, we would like to apply for 2 sessions with 24 hours for each session.