## Supplementary Information

***Electrophysiology assay for TAX-4_1G, TAX-4_2G, TAX-4_3G and DCM10***

We generated four TAX-4 mutations, including three glycine-insertion mutations, TAX-4_1G, TAX-4_2G, and TAX-4_3G, and one disease-causing mutation DCM10 (R421W). Whole-cell current recording result (Fig. 1) showed that all these four mutants were unable to respond to a saturated concentration (100 μM) of intracellular cGMP, indicating these mutations decoupled TAX-4 transduction.

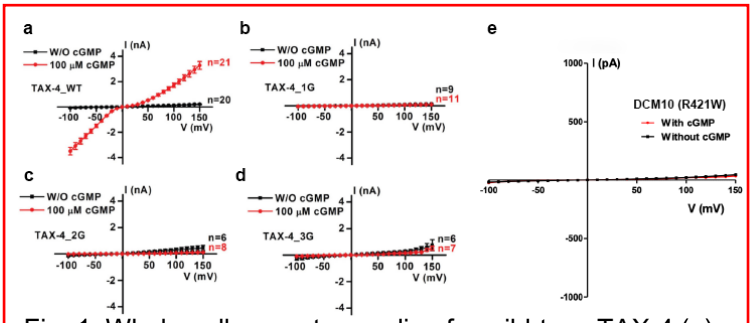


Fig. 1. Whole-cell current recording for wild-type TAX-4 (a), TAX-4_1G (b), TAX-4_2G (c), TAX-4_3G (d) and DCM10 (e) in HEK293T cells.

***Protein purification and amphipol exchange for TAX-4_2G and DCM10***

TAX-4_2G and DCM10 mutant 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 both TAX-4_2G and DCM10 samples are homogenous and good enough for cryo-EM study (Fig. 2).

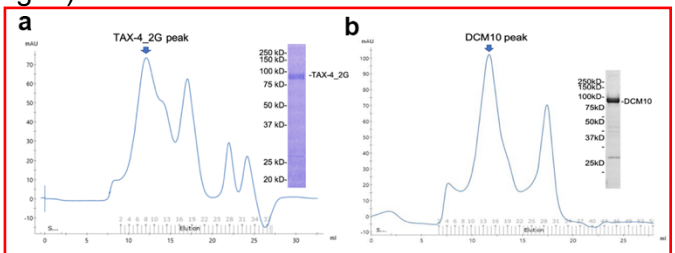


Fig. 2. Gel filtration and SDS-PAGE analysis for TAX-4_2G (a) and DCM10 (b) samples.

***Image acquisition on F20 and processing for TAX-4_2G and DCM10***

We prepared cryogenic grids using vitrobot machine (FEI) for TAX-4_2G and DCM10 proteins, and collected a 648-micrograph dataset and a 943-micragh dataset for TAX-4_2G and DCM10 respectively 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 both TAX-4_2G (Fig. 3b) and DCM10 (Fig. 4b). Through 3D refinement and post-processing, we were able to refine TAX-4_2G map to 5.1 Å resolution (Fig. 3c) and DCM10 to 4.03 Å resolution (Fig. 4c).

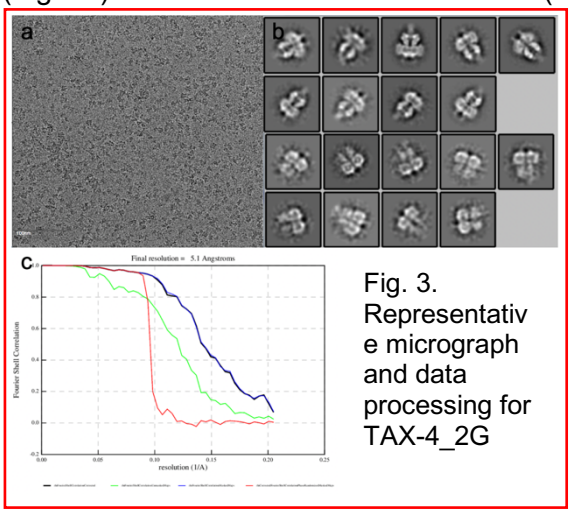


Fig. 3. Representative micrograph and data processing for TAX-4_2G

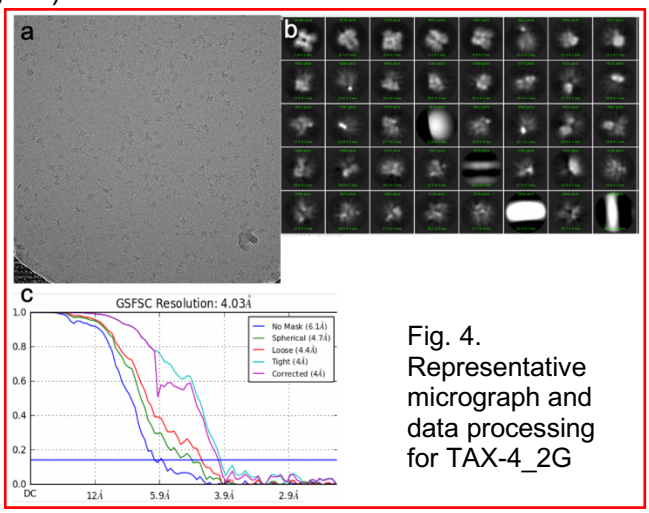


Fig. 4. Representative micrograph and data processing for TAX-4_2G

***Grid availability and session request***

We already have back-up grids for TAX-4_2G and DCM10. 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.