

Supplementary Information (for proposal submission):

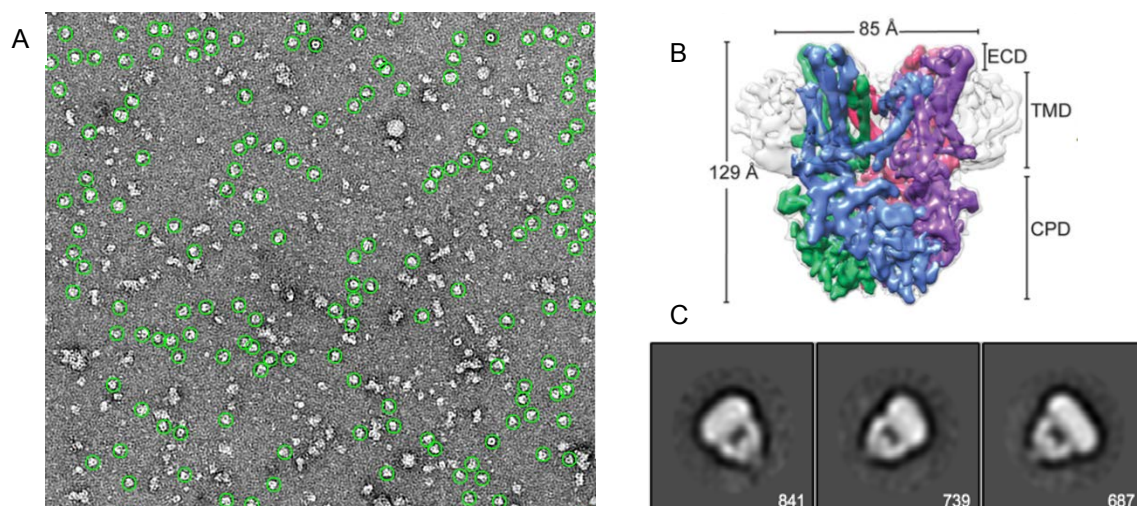


Figure 1. Representative negative stain two-dimensional class averages of the TRPC3 *Mwk* mutant. TRPC3 was prepared in 0.1% digitonin and applied 3 μ L to glow discharged grids and absorbed for 30sec. The sample stained using 0.75% (w/v) uranyl formate. Particles from 81 micrographs collected from FEI Tecnai F20 at x50000 magnification and -1.5 μ m defocus were masked in 100x100 pixel boxes and extracted at 256Å. *A*, representative micrograph with particles in green circles. *B*, electron density map of wild-type full-length TRPC3 in GDN (Sierra-Valdez et al. *J. Bio. Chem.* 2018). *C*, representative class averages. 100 classes were generated from 12,205 particles (2,267 are shown above) using 10 iterations. All data processing was done in RELION 3.0.

The negative stain analysis provided an overview of the TRPC3 *Mwk* mutant structure with a well-defined C-terminal domain (CPD) and a flexible transmembrane domain (TMD). Compared to class averages collected in GDN (data not shown), the presence of digitonin facilitates the TMD to adopt a more compact conformation. In addition to wide-type like symmetric architecture (Fig. 1B, C), we observed asymmetrical distribution of electron density in the TMD (Fig. 1C) in the *Mwk* mutant where one side of the TMD contains higher electron density than the other side. The asymmetrical distribution is hypothesized to contribute to an altered channel gating and function. However, due to the lack of molecular details at limited resolution in the negative stain results, we cannot further characterize the TMD conformation of the TRPC3 *Mwk* mutant. Future studies will determine the cryo-EM structure of the TRPC3 *Mwk* mutant which allows us to determine the conformational changes in the TMD and further elucidate the channel gating mechanism.

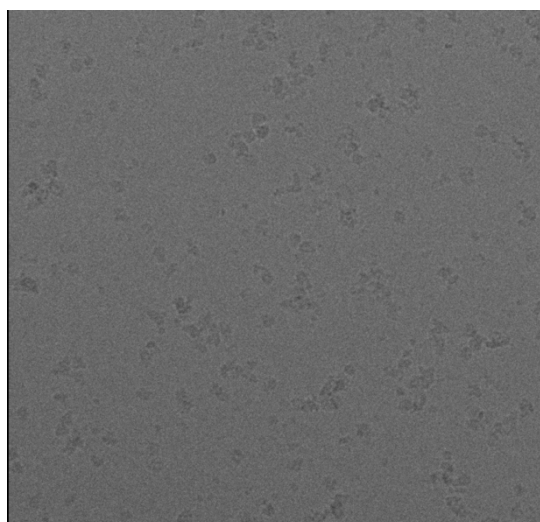


Figure 2. Representative micrograph of TRPC3. TRPC3 was prepared in 0.1% digitonin and applied 2 μ L to the glow discharged 200-mesh 2/1 C-flat grids before plunging into liquid ethane. Initial screening was done on FEI Tecnai F20 equipped with a 4kx4k Gatan Ultrascan CCD camera. Images were collected at x50000 magnification and -3.5 μ m defocus.