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Supplemental information

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Activation of TMEM16F by Inner Gate Charge Mutations and Mechanisms of Lipid and Ion Permeation

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Supplementary Tables

Table S1. Summary of atomistic simulations of TMEM16A

Systems	Mutations	Initial Structure	Length (μs)
sim 1-3	wt	6qp6	1.0 each
sim 4-6	F518K	6qp6	2.5 each
sim 7-9	Y563K	6qp6	2.5 each
sim 10-12	F518KY563K	6qp6	4.0 each
sim 13-15	wt	*sim 10*, 4 μs	1.0 each
Meta 1 and 2	wt	*sim 13,* 150 ns	0.4 each
US 1 and 2	wt	*sim 10*	0.04 per window (0.44 total for each UM run)
US 3 and 4	F518KY563K	*sim 10*	0.04 per window (0.44 total for each UM run)

Table S2. TM helix tilt angles in the closed (wt) and open (F518K/Y563K) states of TMEM16F. All average values and standard deviations were calculated from last 500 ns of sim 1-3 (wt) or sim 10-12 (F518K/Y563K).

	TM1	**TM2**	**TM3**	**TM4**	**TM5**
wt	26.2±2.4	5.7±2.5	42.5±2.5	38.0±2.2	18.4±1.8
F518K/Y563K	26.1±3.8	7.3±3.5	42.1±3.3	39.6±3.6	18.2±3.3
	TM6	**TM7**	**TM8**	**TM9**	**TM10**
wt	35.9±1.2	33.7±2.0	32.2±1.3	11.8±2.3	17.5±2.3
F518K/Y563K	36.2±1.7	33.6±2.4	31.6±1.9	13.2±1.4	17.6±3.4

Supplementary Movies

Movie S1: Spontaneous pore opening of the Ca^{2+}-bound mTMEM16F F518K/Y563F mutant. The movie was generated based on *sim10*. Chain A is shown in the front view by cartoons with TMs 3-6 colored in yellow, red, blue and green, respectively, and the other TMs in cyan. The phosphate atoms in lipid headgroups are represented as transparent orange spheres. The two bound Ca^{2+} ions are shown as two red spheres. The inner gate residues, K518, K563 and I612, are shown as yellow sticks. Note that, as the pore opens up, it becomes fully occupied with lipid headgroups.

Movie S2: A spontaneous lipid translocation event observed in mTMEM16f F518K/Y563K mutant simulation (*sim 12*). Chain A is shown in the front view. TMs, phosphate atoms and inner gate residues are shown in sticks and colored according to atom types. The phosphate atoms of the passing lipid are shown with larger orange spheres, while the tail is shown in stick.

Movie S3: A representative ion permeation event sampled during metadynamics simulations of wt protein of mTMEM16F. Chain A is shown in the front view and elements, such as TMs, phosphate atoms and inner gate residues, are presented with the same scheme as in Movie S1. The passing chloride ion is shown as a green sphere along with its hydration shell. Note that the ion resides in the intracellular and extracellular vestibules for extended periods before and after crossing the hydrophobic inner gate region (the major free energy barrier).

Supplementary Figures

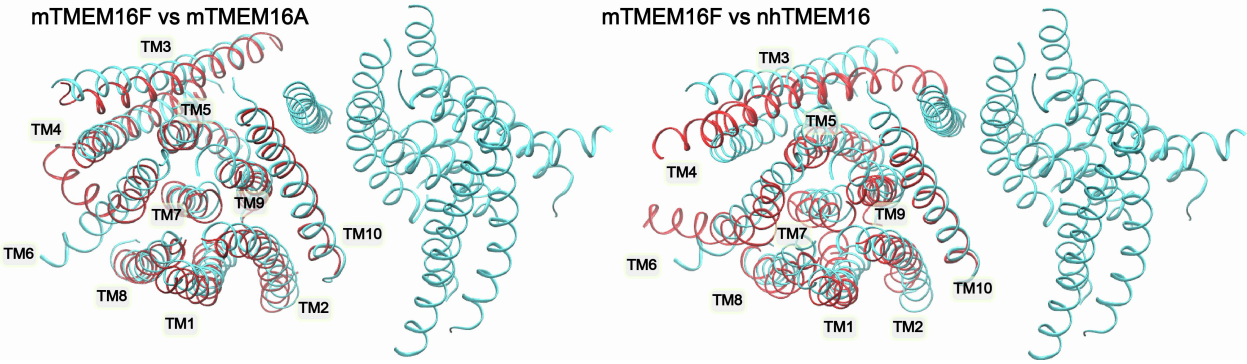

Fig. S1 Comparison of TM arrangement between mTMEM16F and other TMEM16 proteins. Top views of the Ca^{2+}-bound mTMEM16F (PDB: 6QP6, cyan cartoon,) superimposed on the Ca^{2+}-bound TMEM16A (PDB: 5OYB) (left, red cartoon) and the Ca^{2+}-bound nhTMEM16 (PDB: 4WIS) (right, red cartoon). Both structural alignments are based on the backbone of TM1-3 and TM7-10.

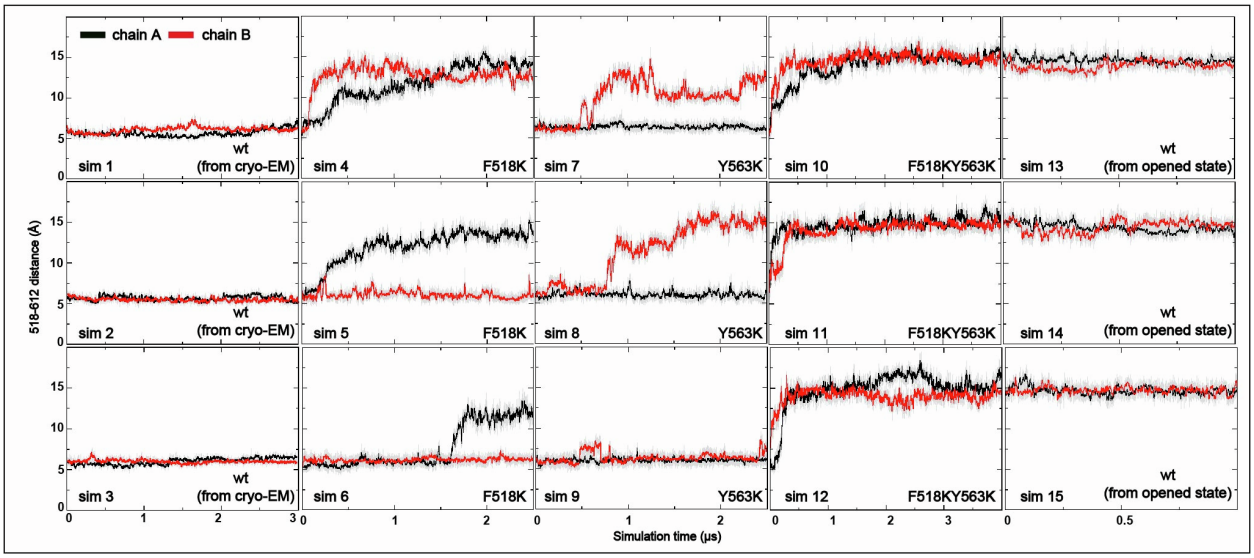

Fig. S2 Cα distance between inner gate residues 518 and 612 in various simulations (Table S1). All traces plot 2.5 ns (50 frame) running averages for clarity and the raw data (recorded every 50 ps) are represented as gray lines.

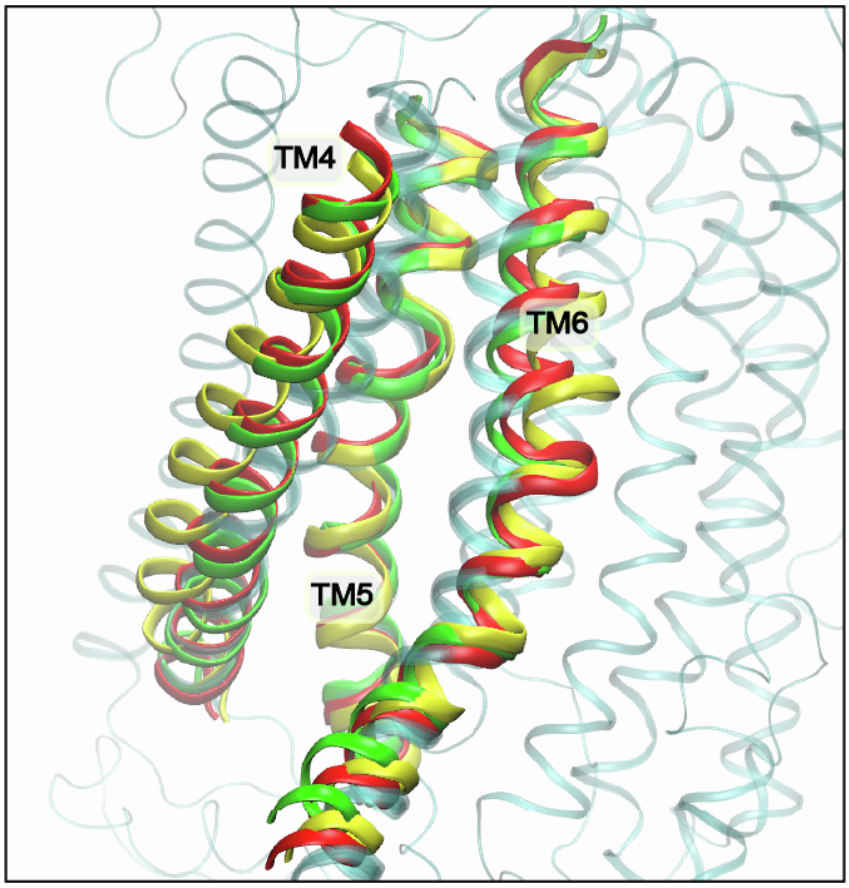

Fig. S3 Superposition of the pore region in representative fully open states of F518K (red), Y563K (green) and F518K/Y563K (yellow) mutants. The last snapshots from trajectories *sim* 4 (chain A), 8 (chain A) and 10 (chain A) are used, as they represent the most dilated pore conformations sampled for each mutant (see Fig. S2).

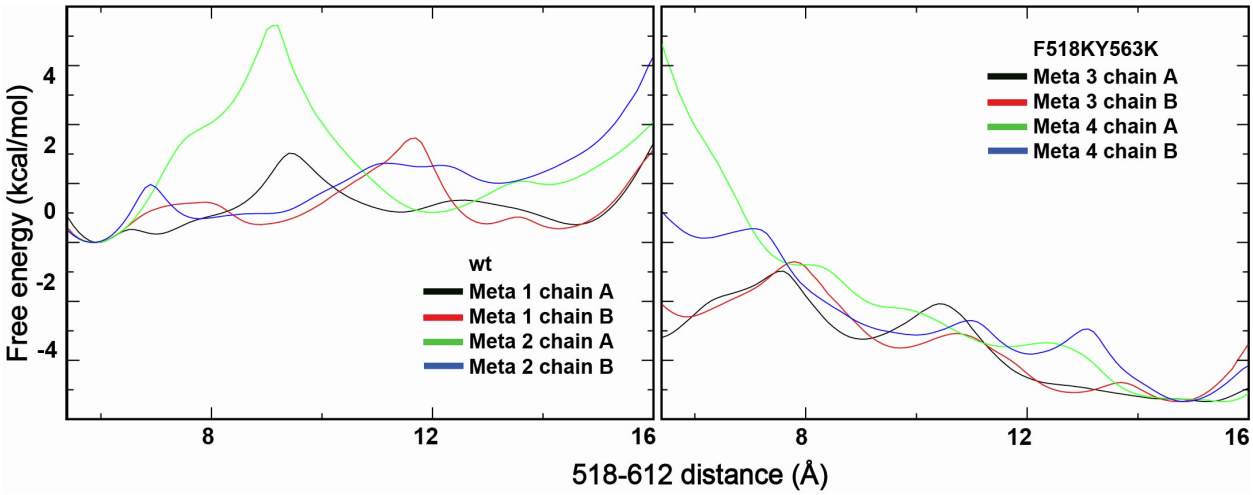

Fig. S4 Individual free energy profiles of pore opening of the mTMEM16F wt and F518K/Y563K mutant along the distance between Cα atoms of inner gate residues 518 and 612 (Table S1).

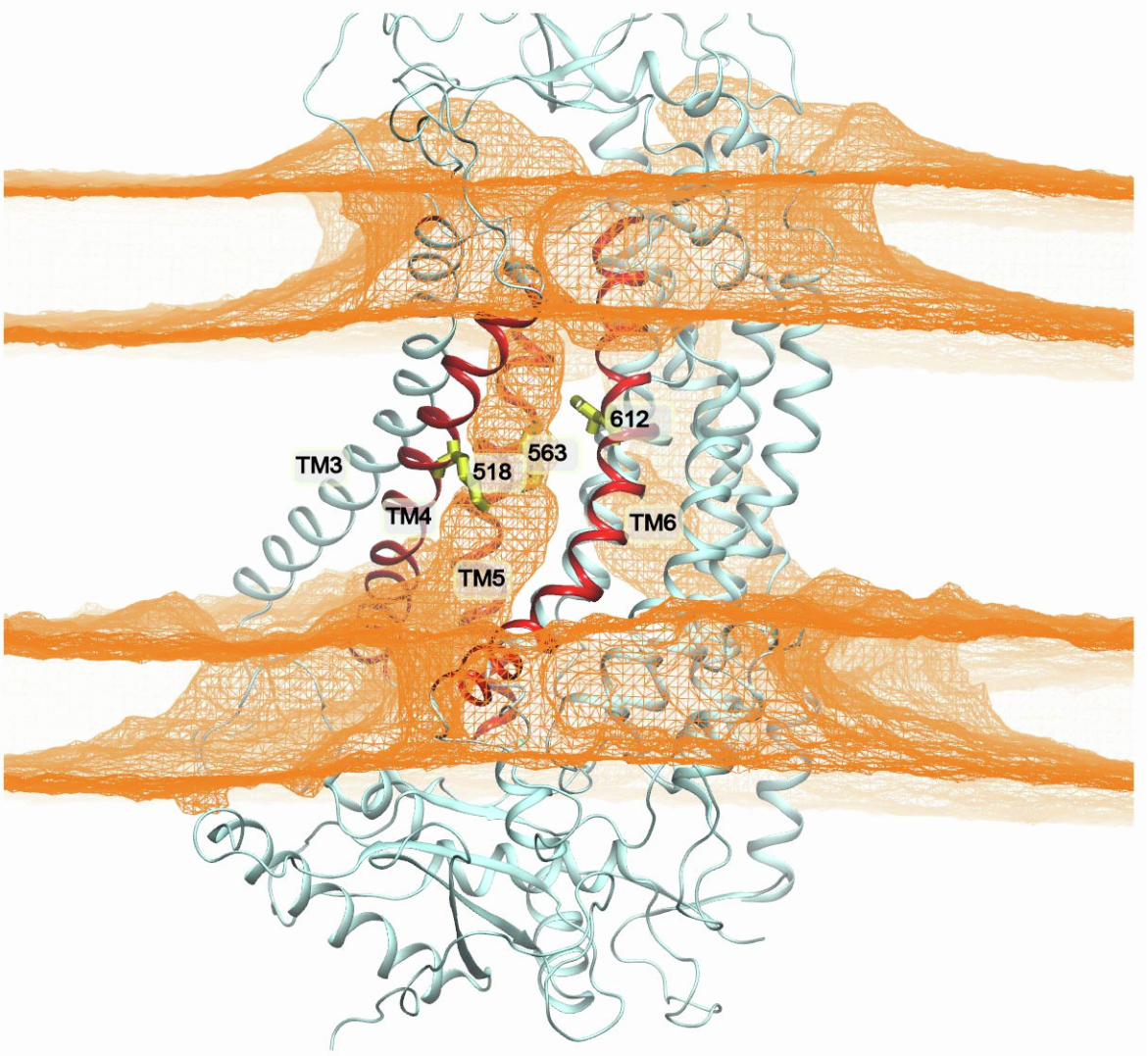

Fig. S5 The 3D density map of lipid phosphate group. Isovalue equals to 0.001 atom/Å^3. The lipid density map was calculated by using 2-4 μs segment of the trajectory from *sim 10*.