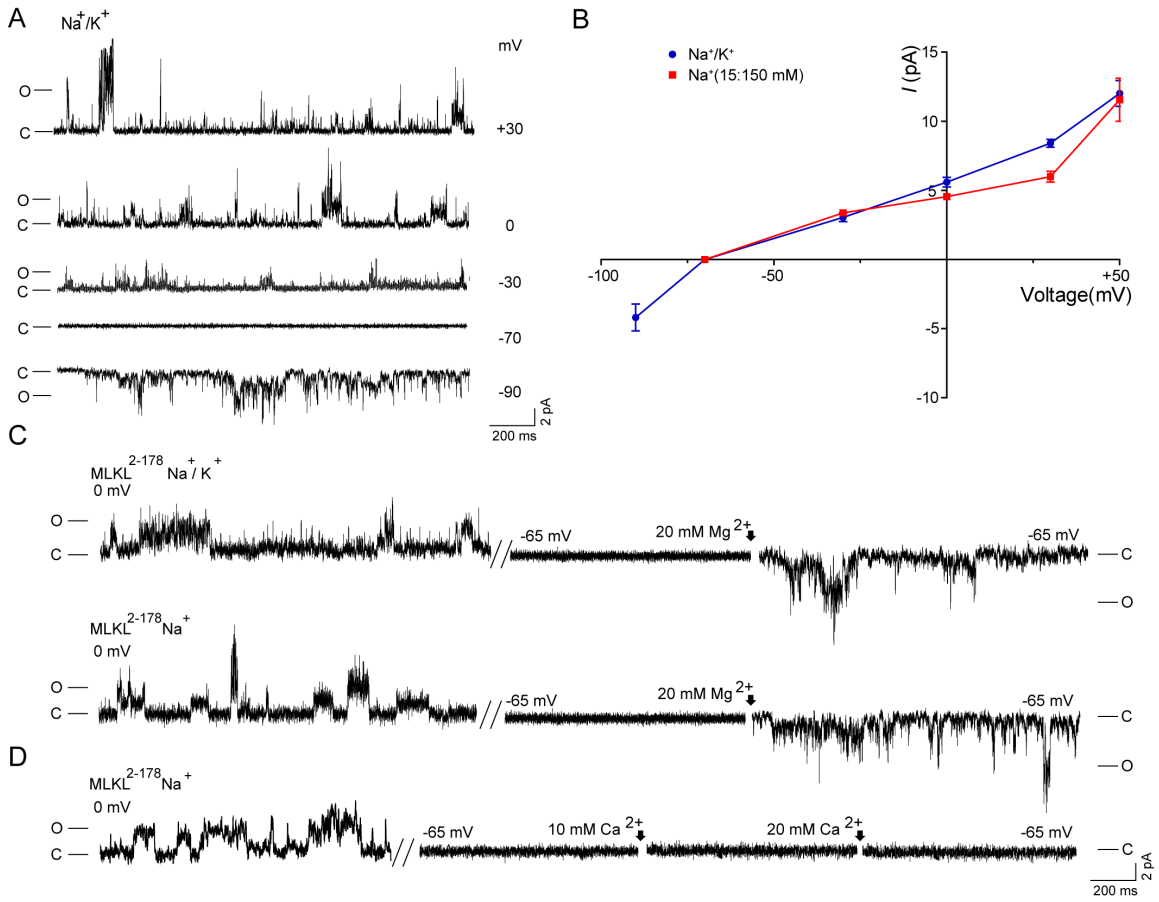

Figure S2. MLKL N-terminal domain (MLKL$^{2\text{-}178}$) functions similar with full length MLKL$^{E/D}$. (A) Representative traces of MLKL$^{2\text{-}178}$ in Na^+/K^+ mixture solutions under indicated membrane potentials. For the Na^+/K^+ mixture solutions, the *cis* side solutions contain 10 mM Na^+ and 5 mM K^+. The *trans* side solutions contain 120 mM Na^+ and 30 mM K^+. (B) Current-voltage plots of MLKL$^{2\text{-}178}$ channels in the indicated solutions. The asymmetric Na^+ solutions contain 15:150 mM (*cis*:*trans*) Na^+. The reversal potentials in the two types of solutions are approximate -65 mV, indicating a cation selectivity of MLKL$^{2\text{-}178}$ channels. （C）A similar strategy shown in Figure 3 was performed to investigate

Mg^{2+}permeability of $MLKL^{2-178}$. $MLKL^{2-178}$ mediates typical step-like currents in the presence asymmetric Na^+ or Na^+/K^+ solutions. The frequent and continuous outward currents indicate that the $MLKL^{2-178}$ channels were incorporated into the membranes. The membrane potential was then changed to the corresponding equilibrium potential, -65 mV, to eliminate the currents. Inward step-like signals appeared when 20 mM (final concentration) Mg^{2+} was added to the *cis* side, indicating presence of Mg^{2+} currents ($n > 4$). (D) MLKL2-178 cannot induce currents in Na^+/Ca^{2+} mixture solutions. (E) A similar strategy shown in Figure 3 was performed to investigate Ca^{2+}permeability of $MLKL^{2-178}$. Representative step-like currents in the asymmetric 15:150 mM (*cis*:*trans*) Na^+ solutions indicate the formation of $MLKL^{2-178}$ channels in the lipid membranes. Next, the holding potential was changed to -65 mV, the Na^+ equilibrium potential, to eliminate the Na^+ signals. Additional supplementation of 10 mM and 20 mM (final concentration) Ca^{2+} was added to the *cis* side sequentially.